JOURNAL OF AGRICULTURAL RESEARCH

VOL. 43 WASHINGTON, D. C., AUGUST 1, 1931 No. 3

FURTHER STUDIES ON TOBACCO RING SPOT IN VIRGINIA[1]

By R. G. HENDERSON, *Assistant Plant Pathologist*, and S. A. WINGARD, *Plant Pathologist, Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station*

INTRODUCTION

Since 1917 the virus disease of tobacco known as ring spot has attracted considerable attention in practically all of the important tobacco-growing districts in the United States and in a number of other countries. Numerous reports by workers in widely separated parts of the world indicate that this disease is widespread and present to some extent wherever tobacco is grown. H. R. Angell, senior plant pathologist of the Council for Scientific and Industrial Research, Canberra City, Australia, in correspondence on February 24, 1930, stated that tobacco ring spot was assuming economic proportions in the tobacco-growing districts of that country. In some fields 30 per cent of the plants were reported to be infected. In 1926, Moore (*11*)[2] published an article in the Journal of the Department of Agriculture, South Africa, in which he illustrated a disease of tobacco not unlike ring spot. Moore, however, classified this trouble as a disease of unknown cause. Ring spot was reported from Sumatra in 1926 by Jochems (*6*), and by Butler (*1*) from Nyasaland in 1928. The reports of ring-spot occurrence in the United States are so numerous and familiar to workers on the subject that they will not be enumerated here.

Ring spot is widespread in Virginia and is probably causing a greater loss than past reports have indicated. In Washington County 95 per cent of the acreage in 1929 showed ring-spot infection. In some fields as many as 90 per cent of the plants were infected. The average infection was estimated at 8 per cent and the decrease in yield about 1 per cent of the total crop in that county. The disease was known to be present throughout the State, but the surveys in counties other than Washington in 1929 were too general to give accurate figures as to the percentage of infection.

In the 1930 survey, ring spot was observed on seedling plants in the bed. A survey of 104 beds located in Washington, Prince Edward, Buckingham, Pittsylvania, Amelia, Mecklenburg, Bedford, and Appomattox Counties showed 8 beds with ring-spot infection, 6 of which were in Washington County, 1 in Pittsylvania, and 1 in Prince Edward. The number of infected plants in each bed was small, usually not more than two or three. On first thought it would seem from these data that ring spot was more prevalent in Washington County, but this is not necessarily true. Contrary to the methods followed in the other sections of the State, in Washington County it is

[1] Received for publication Feb. 26, 1931; issued August, 1931. Paper No. 77 from the Department of Botany and Plant Pathology, Virginia Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 206.

a common practice to locate the beds in the open and very often on old bed sites or near fields where tobacco has been previously grown. Sterilization of the soil by burning, however, is a usual precaution. The one bed in Prince Edward County showing ring spot was on an old bed site. Considering the above data from the standpoint of plant-bed location, it would seem that the source of infection in the beds was closely tied up with the previous tobacco crop grown on or near the bed site.

The 1930 field survey showed ring spot to be present in practically every section of the State. Out of 480 acres of tobacco located in nine different counties, 229, or 47 per cent, showed ring spot. The percentage of acreage infection was about the same as for the previous year, but the percentage of plants infected was less. The estimated infection in Washington County in 1930 was 5 per cent as compared with 8 per cent in 1929.

This paper is a presentation of the data obtained from laboratory and greenhouse experiments carried out in an effort to determine some of the properties of the virus causing ring spot.

MATERIALS AND METHODS

The tobacco ring-spot virus used in these experiments was originally secured from commercial plantings in Virginia. Transfers were made at frequent intervals to healthy Turkish tobacco plants growing in the greenhouse.

Unless otherwise stated the tobacco plants used in the various experiments were of the Turkish variety. In making the inoculations, the leaves were swabbed with a cotton plug saturated with the inoculum. This method was used by Wingard (*15*) and found to be very satisfactory.

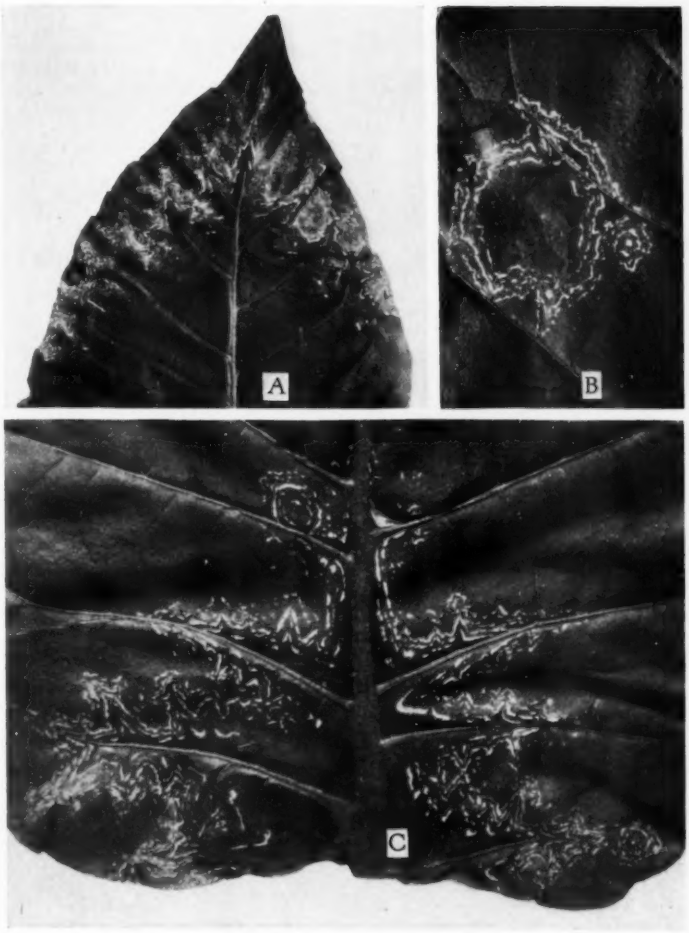
The first sign of infection following artificial inoculation occurs as a type of local infection on the leaves receiving the inoculum. Therefore, the number of local lesions developing on a leaf receiving a definite amount of inoculum could be used to indicate the virulency of the virus. Advantage was taken of this fact in comparing the virulency of the virus in different juices and solutions.

NATURAL OCCURRENCE OF SIMILAR OR LIKE VIRUSES ON HOSTS OTHER THAN TOBACCO

SWEETCLOVER

Natural infection of sweetclover (*Melilotus officinalis* Lam.) with a virus that produces ring-spotlike symptoms on tobacco was reported by Wingard (*15*) in 1928. Since that time the investigation has been continued to determine whether or not the disease occurring on sweetclover is caused by the tobacco ring-spot virus. In a few cases in Washington County where tobacco followed sweetclover in the rotation, ring spot was especially severe. This suggested that sweetclover might be a natural host and thereby responsible for the high percentage of ring-spot infection in these fields.

During the summer of 1929 several abnormal sweetclover plants were collected on the college farm and used to inoculate small healthy Turkish tobacco plants growing in the greenhouse. Out of 18 inoculations, 9 produced ring-spotlike symptoms in five to eight days. The symptoms appeared first as small brown flecks around which later developed light-colored or necrotic rings. Often the rings were



A DISEASE ON TURKISH TOBACCO PRODUCED BY A VIRUS FROM NATURALLY INFECTED SWEETCLOVER

A, Leaf taken from upper part of plant showing the symptoms approaching the leaf apex (about 1/10 X); B, a portion of a leaf illustrating the definite concentric rings (natural size); C, leaf taken from middle of stalk showing zigzag lines near the leaf base (natural size).

A. Rings produced on Turkish tobacco following inoculation with virus from yellow ironweed (*Verbesina alternifolia* Britton); B, portion of a leaf showing the infection produced by inoculating with virus from squash. (About natural size)

very faint and the central spot was absent. From these local lesions, systemic infection developed. Systemic infection produced a pattern-like effect on the uninoculated leaves in the top of the plant. Yellowish-brown lines following the veinlets often formed concentric rings like those shown in Plate 1, B. Another expression of systemic infection was the formation of transverse zigzag lines across the leaf. On the leaves at the middle of the stalk the transverse lines were near the base, as shown in Plate 1, C, while the successive leaves above this point showed the zigzag markings progressing toward the leaf tip. Plate 1, A, is a photograph of a leaf taken from the upper part of an infected plant.

In general the symptoms produced on tobacco by the virus from sweetclover are almost identical with those of tobacco ring spot. The only important difference is apparently in the intensity of the symptoms produced. Tobacco plants infected with the sweetclover virus are only slightly dwarfed and the leaves are very mildly distorted; whereas plants infected with the true tobacco ring-spot virus are severely stunted, especially so in the early stages of infection, and many of the leaves are badly distorted and puckered with a large portion of the leaf tissue necrotic.

All symptoms of natural infection on sweetclover plants have not been very well worked out. Dwarfing of the plants with a yellowing of the leaves seems to indicate infection. Leaves from diseased plants held to the light usually exhibit light-colored areas in the tissue and in some cases faint rings, but plants with chlorotic abnormalities in the leaves are not always infected with ring spot. Dwarfing of otherwise apparently healthy plants, however, seems to be a very definite sign of infection. Inoculations on sweetclover plants with both the tobacco ring-spot virus and the sweetclover ring-spot virus have given unsatisfactory results in most instances.

YELLOW IRONWEED

On July 7, 1929, in Washington County, leaves of the commonly occurring weed called yellow ironweed (*Verbesina alternifolia* Britton) were collected near a tobacco field in which considerable ring-spot infection was found. This material was brought into the laboratory and used to inoculate healthy tobacco plants. Nine days later the inoculated leaves showed numerous lesions not unlike an early stage of tobacco ring spot. The rings were about 1 cm. in diameter. A few secondary rings developed around the original lesions but they were faint. (Pl. 2, A.) The plants seemed to outgrow the disease and little development beyond the initial infection resulted. At a later date another specimen of yellow ironweed was collected, and inoculations in the greenhouse gave similar results.

The yellow-ironweed leaves used in these tests were slightly more yellowish than the normal leaves, and the ones near the buds of the infected plants were somewhat distorted, suggesting a rosettelike condition. A few brown spots of indefinite outline were also present.

JIMSON WEED

Late in the summer of 1929, a specimen of Jimson weed (*Datura stramonium* L.) was collected in Washington County near a tobacco field in which a high percentage of the plants were infected with ring

spot. This specimen appeared to be infected with ring spot, according to the symptoms described for Jimson weed by Wingard (*15*). Inoculation on healthy tobacco plants proved that this assumption was correct. Whether or not this weed had become infected from the tobacco or whether it had been a source of infection for the tobacco could not be determined at that time. It seemed evident, however, from the high percentage of infection in the tobacco in this particular field that some weed host must have acted as a source of infection.

CANTALOUPE

During a plant pathological inspection tour in North Carolina in the summer of 1929, the senior author collected leaves from a cantaloupe plant (*Cucumis melo* L.) on a plot adjacent to a tobacco field at the Oxford Tobacco Experiment Station. Faint chlorotic rings in the leaf tissues suggested that this plant was infected with ring spot. Inoculation on tobacco produced symptoms that were identical with true tobacco ring spot. Several transfers have been made in the greenhouse and there is no doubt that this virus is the same as that producing ring spot on tobacco.

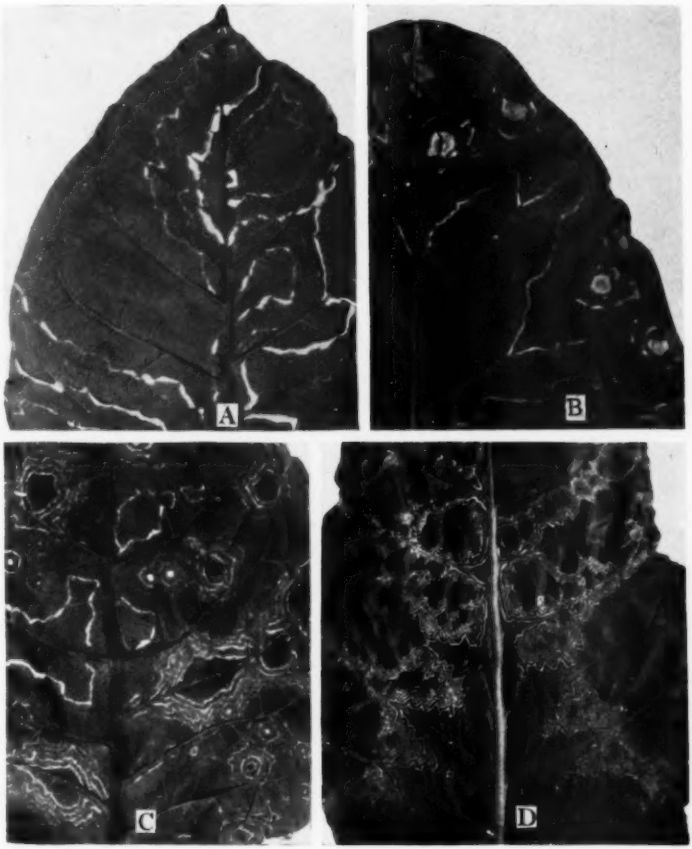
In August, 1930, an acre field of cantaloupes was observed in Prince Edward County, Va., in which 30 or 40 per cent of the plants were infected with ring spot. It was impossible to determine the amount of injury produced by this infection since the severe drought had caused considerable burning of the foliage of all the plants. Plate 5, A, shows a section of a leaf taken from a naturally infected cantaloupe plant.

SQUASH

The leaves of a squash plant (*Cucurbita pepo* L. var. *condensa* Bailey) growing in a garden in Blacksburg were noticed to have light-colored areas in the tissues, which looked much like the symptoms described by Wingard (*15*) for ring spot on cantaloupe, crookneck squash, etc. An inoculation with expressed juice from the squash leaves was made on tobacco. Rings developed on the inoculated tobacco leaves in about five days (pl. 2, B), but the plants seemed to outgrow the disease and no further symptoms developed.

PETUNIA

The natural occurrence of ring-spot infection on petunia (*Petunia violacea* Lindl.) has been reported by Henderson (*5*). At the time of this report only one series of tobacco plants had been inoculated. On the plants in this series the infection was positive and the symptoms produced corresponded in general with those produced by the tobacco ring-spot virus. Later, however, it was noticed that the symptoms were not identical with those of tobacco ring spot. The disease was milder and less destructive. Systemic infection developed from the local lesions on the leaves receiving the inoculum as in the case of tobacco ring spot, but secondary lines and rings were faint or absent. Plate 3, B, illustrates this characteristic. In this illustration the pattern of large necrotic lines is similar to ring spot, yet the secondary lines as seen in the photograph of tobacco ring spot (pl. 3, D) are absent. A comparison of these two figures will show the difference in tobacco ring spot and the petunia virus disease.



SYMPTOMS PRODUCED ON TURKISH TOBACCO BY A VIRUS FROM NATURALLY INFECTED PETUNIA

A, Symptoms produced on the lower leaves by the fourth transfer of the virus; B, type of symptoms produced by the virus in the third transfer on both upper and lower leaves; C, type of symptoms produced by the virus on the upper leaves in the fourth transfer; D, symptoms of systemic ring-spot infection for comparison. (All about natural size.)

OAK-LEAF TYPE OF PATTERN PRODUCED ON A TURKISH TOBACCO LEAF BY THE PETUNIA VIRUS IN THE FOURTH TRANSFER. (SLIGHTLY REDUCED)

The mild symptoms of the petunia "ring spot" remained more or less constant through the first three transfers on tobacco. The lower leaves on the plants inoculated for the fourth transfer also exhibited only the large necrotic lines. (Pl. 3, A.) At a later date in this fourth transfer a more severe type of infection began to develop on the upper leaves. A complicated pattern of necrotic and chlorotic rings and zigzag lines appeared (pl. 3, C) and many of the leaves became distorted. In practically every detail the symptoms corresponded more nearly with those of tobacco ring spot. Plate 4 shows the oak-leaf type of pattern produced by the petunia virus after it had undergone three transfers on Turkish tobacco. This particular pattern is of common occurrence in tobacco ring spot.

OTHER PLANTS STUDIED

A number of other plants occurring around tobacco fields, including pokeweed, hogweed, broadleaf plantain, cocklebur, nightshade, horse nettle, smartweed, lamb's-quarters, and yellow aster, were used to inoculate tobacco to determine whether or not they carried the ring-spot virus. None of these, however, proved to be infected. Pokeweeds on the experiment station farm that had developed the disease from artificial inoculation in the summer of 1928 apparently did not carry the virus in 1929. Tobacco plants inoculated in 1929 with the juice from the roots and leaves of these plants failed to develop ring-spot symptoms.

PROPERTIES OF THE VIRUS

REACTION OF THE VIRUS TO HEAT

In an attempt to determine the thermal death point of the tobacco ring-spot virus, the following experiments were made: Juice was expressed from ring-spot-infected tobacco plants and strained through a double thickness of cheesecloth to remove the coarse particles of plant tissue. A number of small thin glass vials were fitted with corks, and 1.5 c. c. of the undiluted juice placed in each. The vials containing the virus juice were then divided into five groups, *a*, *b*, *c*, *d*, and *e*. A large water vat equipped with steam-pipe connection for heating the water was used to maintain a constant-temperature bath. The vials were exposed in the water bath to a temperature of 70° C. for a period of time varying with each of the groups. Group *a* was exposed 5 minutes; *b*, 4 minutes; *c*, 3 minutes; *d*, 2 minutes; and *e*, untreated, was held as a check. Forty-five seconds were allowed for the juice to reach the temperature of the bath. The vials containing the juice were instantly plunged into cold water following the exposure to heat. After this treatment the solution in each vial was used to inoculate a small healthy tobacco plant in order to determine whether or not the virus had been inactivated by the exposure. This experiment was repeated at a temperature of 60° and the time allowed for the juice to reach the bath temperature was decreased to 30 seconds.

The results of these experiments are given in Table 1. In 1½ minutes at 70° C. the infecting power of the virus was somewhat reduced, in 2 minutes its virulence was materially reduced, and in 3 minutes the virus was completely inactivated. At 60° C. the virulence of the virus was reduced considerably even with short exposure, but after an exposure of 10 minutes the juice was still infectious.

TABLE 1.—*Effect of exposure to heat upon the virulence of the ring-spot virus*

Period of exposure	Temperature	Plants inoculated	Leaves inoculated	Plants infected	Leaves infected	Total local lesions produced
	° C.	*Number*	*Number*	*Number*	*Number*	*Number*
5 minutes	70	5	15	0	0	0
4 minutes	70	5	15	0	0	0
3 minutes	70	4	16	0	0	0
2 minutes	70	4	16	4	8	20
1½ minutes	70	4	16	4	14	80
10 minutes	60	4	16	2	5	9
8 minutes	60	4	16	4	8	24
6 minutes	60	4	16	4	9	30
4 minutes	60	4	16	4	10	43
2 minutes	60	4	16	4	12	65
Check		4	16	4	16	170

REACTION OF THE VIRUS TO SUBZERO TEMPERATURE

As shown above, the ring-spot virus is inactivated in about 3 minutes at 70° C., and in about 5 to 10 minutes at 60° C. It is also known to become inactivated in 12 to 24 hours at ordinary room temperature. Heat, therefore, is very destructive to the ring-spot virus in expressed plant juice. On the other hand, freezing acts as a preservative. Samples of tobacco and petunia juice containing the ring-spot virus were placed in cold storage at a temperature of −18° C. on February 11, 1928, and the virus was still virulent when tested on tobacco on December 15, 1929. This shows that the virus may retain its virulency for at least 22 months when kept at a temperature of −18° C. The last of the stored virus was used in the test on December 15, 1929; consequently further inoculation tests could not be made. It is evident, nevertheless, that the virus would have remained virulent longer than 22 months at this temperature.

EFFECT OF DESICCATION ON THE VIRUS

The ring-spot virus is very sensitive to desiccation. The writers have never succeeded in getting infection from dry material, no matter how fresh it happened to be. Dry infected tobacco leaves have been tried repeatedly but always with negative results. This no doubt explains why Fromme and Wingard (*4*) failed to get infection from artificial inoculation in their first studies. They depended upon material which had been sent in from the field as a source of inoculum, and in most cases it had dried out in transit.

PRECIPITATION OF THE VIRUS WITH CHEMICALS

Some of the methods used by Vinson and Petre (*14*) in precipitating the tobacco mosaic virus were tried on the ring-spot virus. Preliminary experiments indicated that safranine either destroyed the virus or was unable to precipitate it. When this reagent was used both picric acid and amyl alcohol were employed in an attempt to free the virus from the safranine precipitate. Since the results of this preliminary work did not appear promising, further experimenting was not undertaken.

The outline given on page 197 was followed in precipitating the ring-spot virus with acetone.

To 50 c. c. of expressed juice, add 2 c. c. of basic lead acetate solution (200 gm. per liter); place in cold storage for several hours; thaw, and centrifuge or filter.

- To the filtrate add 3 c. c. of barium acetate solution (2 c. c. of N acetic acid to 5 c. c. saturated solution barium hydroxide); place in cold storage for several hours; thaw, and centrifuge or filter.
  - Discard residue.
- To the cold filtrate add 2 volumes of acetone at −20° C. Let stand for 10 to 15 minutes; filter with partial vacuum through Whatman filter No. 42.
  - Discard residue.
- To the residue on filter paper, add 50 c. c. distilled water.
  - Discard residue.
- Use the filtrate to inoculate plants.
  - Discard remainder of residue.

The acetone was added to the juice in the cold-storage room at a temperature of about −2° C. The juice was slightly above the freezing point and acetone was about −20° C. A brownish-white flocculent precipitate formed quickly upon the addition of the acetone to the juice, and in most cases settled to the bottom of the container very readily. This precipitate was removed by filtering through a Whatman filter paper No. 42 with the aid of a suction pump. A quantity of water equal to that of the original volume of juice was added to the filter paper and the filtrate thus obtained was then used to inoculate plants. Only a small amount of brownish sediment remained on the paper.

This treatment reduced the virulence of the virus somewhat but did not destroy its infective power. This experiment has been repeated a number of times with the same results. Because of this reduction in virulence, the development of systemic infection was delayed. The reaction to acetone precipitation of the virus from sweetclover, as well as that of the virus from apparently healthy potato described by Johnson (*8*), was compared to that of the tobacco ring-spot virus. The virulence of the sweetclover virus was reduced to about the same extent as the tobacco ring-spot virus, but the virus from potato did not seem to be affected in the least. Table 2 shows this comparison.

TABLE 2.—*Effect of precipitation with acetone upon the virulence of different viruses* [a]

Virus type	Effect of inoculating 5 tobacco plants with—					
	Virus precipitated with acetone		Juice frozen and centrifuged		Juice untreated	
	Local infection	Systemic infection	Local infection	Systemic infection	Local infection	Systemic infection
Tobacco ring spot	5 plants, mild.	None	5 plants, moderate.	3 plants, moderate.	5 plants, severe.	5 plants, severe.
Sweetclover ring spot.	5 plants, very mild.	1 plant, slight.	5 plants, mild.	4 plants, mild.	5 plants, moderate.	5 plants, moderate.
Potato ring spot	5 plants, moderate.	5 plants, moderate.	5 plants, moderate.	5 plants, moderate.	do	Do.

[a] Notes made 12 days after inoculations were made.

Ninety-five per cent ethyl alcohol was used in the place of acetone with similar results except that the precipitate did not form so readily as with acetone.

FILTRATION THROUGH BERKEFELD FILTERS

Priode (*12*) attempted to filter the ring-spot virus through a grade N Berkefeld filter but was unable to obtain infection from the filtrate. The writers' first trial in filtering ring-spot-infected juice was somewhat unsatisfactory. Expressed juice strained through a double thickness of cheesecloth was diluted with an equal volume of distilled water, and filtered under reduced pressure, three grades of Berkefeld filters being used, W, N, and V. The organic matter in the expressed juice gummed up the pores of the filters to such an extent that filtration was slow. About an hour was required to secure enough filtrate (about 10 c. c.) to inoculate four tobacco plants. No infection was secured from inoculations made with these filtrates. This filtration test, however, was repeated with undiluted juice that had been more thoroughly purified of plant material by filtering through a fine grade of filter paper. This time the juice filtered more readily through all three grades of filters. Inoculations made with the filtrates showed that the virus had passed the V grade filter but not the finer ones.

At a later date, it was desired in an experiment to have an infectious juice that was free of bacteria and fungi. Filtration through Berkefeld filters was again tried. This time the expressed juice was freed of plant tissue by treatment with lead acetate and barium acetate as outlined above in the acetone-precipitation experiment. The juice thus purified was filtered through grades N, V, and W into sterile flasks. A portion of the filtrate was then allowed to stand for several days to see if any bacterial growth would develop. All three filtrates proved to be absolutely sterile. Another portion of these filtrates had been used to inoculate healthy tobacco plants. Notes were taken on the amount of infection nine days later. The results are shown in Table 3. The amount of virus was materially reduced by all three filters, especially the W grade. The virus, however, was apparently not attenuated, the incubation period only being prolonged. With the filtrate from the W filter, systemic infection was secured on one plant only and the local infections were either absent or so faint that they were unnoticeable.

TABLE 3.—*Effect of filtration through Berkefeld filters of different grades upon the virulence of the ring-spot virus*

Filter	Plants inoculated	Leaves inoculated	Plants developing local infection	Leaves infected	Local lesions produced	Plants developing systemic infection
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
W grade	4	16	0	0	0	1
N grade	4	16	2	4	8	2
V grade	4	16	4	14	35	4
Check	4	16	4	16	153	4

THE EFFECT OF DILUTION ON RING-SPOT VIRUS

In the various inoculation studies with tobacco ring spot, the expressed juice from ring-spot-infected leaves was usually diluted with either tap or distilled water before it was used to inoculate healthy plants. To determine how much dilution the virus-infected juice would stand and still produce infection, healthy tobacco plants were inoculated with infected juice diluted with distilled water 1 to 10, 1 to 100, 1 to 1,000, 1 to 10,000, 1 to 100,000, 1 to 1,000,000, 1 to 10,000,000, 1 to 100,000,000, and 1 to 1,000,000,000. On the plants inoculated, there was a gradual decrease in the severity of infection as the dilution increased. Only slight ring-spot infection was obtained with the 1 to 1,000 dilution, and the dilution of 1 to 10,000 gave only a trace of infection and this was obtained in only one instance. The ordinary tobacco mosaic virus used under identical conditions gave infection in dilution as high as 1 to 10,000,000.

THE PERSISTENCE OF RING-SPOT VIRUS IN TOBACCO PLANTS

It has been stated elsewhere on several occasions that tobacco plants seem to develop an immunity to the ring-spot disease. Local lesions develop in 2 or 3 days after inoculation, and in 10 to 14 days lesions appear on the uninoculated leaves as a result of systemic infection. The first symptoms of systemic infection are very prominent, but as new leaves are formed the symptoms which develop on them gradually become less conspicuous until finally there is no evidence of infection on the uppermost leaves.

To determine how long the ring-spot virus would persist in plants after the symptoms had become completely masked, the following experiment was made. Twelve Turkish tobacco plants were inoculated on January 15, 1928. Typical ring-spot symptoms appeared on these plants in 3 days, symptoms from systemic infection appeared as usual in about 10 days, but the leaves which were formed 3 weeks after the date of inoculation were apparently normal and remained free of ring-spot symptoms. Cuttings were made from the top growth of these plants, but the new growth of the cuttings failed to develop ring-spot symptoms even when reinoculated with ring-spot virus from newly infected tobacco plants. The juice from these apparently healthy leaves, however, proved to be very infectious when used to inoculate healthy tobacco plants. These cutting plants in turn had cuttings taken from them to continue the life of the original plants, until finally the ring-spot symptoms had been masked for a year. The virus, however, apparently did not lose any of its virulency during this time.

THE DEVELOPMENT OF RING-SPOT SYMPTOMS ON TOBACCO CUTTINGS AND DETACHED LEAVES

On May 20, 1927, cuttings were taken from plants of four different species of tobacco and placed in flasks containing tap water. The top 10 inches of plants about 2 feet high were used as cuttings. The leaves of these cuttings were inoculated with ring-spot virus on the same day that the cuttings were made. Local symptoms of ring spot appeared in seven days, but they never became very conspicuous, due perhaps to the weakened condition of the cuttings. Some of these

cuttings, nevertheless, developed fairly vigorous root systems and lived for several weeks.

It has been found that ring-spot symptoms may be produced on detached tobacco leaves under certain conditions. On January 18, 1928, 16 Turkish tobacco leaves were removed from the plants, inoculated with ring-spot virus, and placed in moist chambers in the greenhouse. Eight of these leaves revealed definite ring-spot symptoms when examined on January 23. The usual type of primary and secondary rings occurred on these leaves. Symptoms representing later stages of infection, however, failed to develop, owing to the early decay of the leaves.

In another experiment the tops of several plants carrying the upper five or six leaves were removed, and after the leaves had been inoculated with ring-spot virus the cuttings were placed in moist chambers. Definite ring-spot symptoms appeared on the leaves of these cuttings within five days after inoculation.

THE EFFECT OF AGE UPON THE SUSCEPTIBILITY OF TOBACCO LEAVES TO RING-SPOT INFECTION

Observations from the beginning of these studies have indicated that tobacco leaves of medium age are more susceptible to ring-spot infection than either the very old or the very young ones. In order to determine the accuracy of these observations, inoculations were

TABLE 4.—*Effect of age of tobacco plant leaf upon its susceptibility to ring-spo infection*

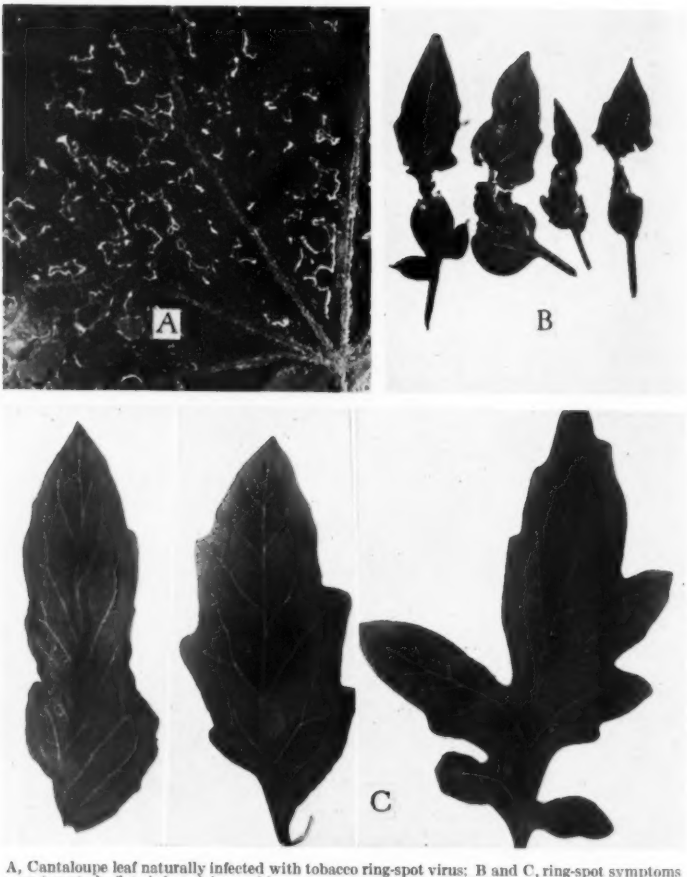
[Tobacco plants inoculated on January 16, 1928, at 4 p. m.]

Plant No.	Leaf No.[a]	Width of leaf	Number of lesions on—				
			Jan. 19	Jan. 20	Jan. 21	Jan. 23	Jan. 24
		Cm.					
1	1	9.5	8	8	18	19	19
	2	8.7	43	43	43	43	43
	3	6.5	2	6	23	23	23
	4	5.5	0	12	23	27	27
	5	3.0	0	4	6	12	12
	6	1.5	0	0	0	25	25
	7	0.5	0	0	0	3	30
	8	(b)	0	0	0	3	3
	9	(b)	0	0	0	0	3
2	1	7.8	85	85	85	85	85
	2	7.5	29	29	29	29	29
	3	6.2	23	23	27	27	27
	4	3.7	0	12	12	12	12
	5	2.6	0	0	0	0	7
	6	1.0	0	0	0	8	21
	7	(b)	0	0	0	1	3
3	1	8.0	0	0	0	0	0
	2	6.9	0	1	2	10	10
	3	6.9	25	25	39	46	46
	4	5.3	29	53	53	53	53
	5	4.2	1	9	12	12	12
	6	2.1	0	0	2	4	12
	7	0.6	0	0	0	6	35
	8	(b)	0	0	0	0	4
4	1	9.0	0	0	0	3	3
	2	8.1	111	111	111	111	111
	3	7.6	141	141	141	141	141
	4	5.4	3	22	27	27	27
	5	4.0	0	4	4	25	25
	6	0.9	0	1	1	17	29
	7	0.7	0	0	0	14	29
	8	(b)	0	0	0	6	6

[a] The leaves were numbered consecutively beginning at the bottom of the plant. [b] Bud leaf.

Index

x



A, Cantaloupe leaf naturally infected with tobacco ring-spot virus; B and C, ring-spot symptoms on tomato leaflets infected by grafting a tomato scion on a tobacco stock and then inoculating the tobacco with the ring-spot virus. (All about natural size)

made on more than one occasion on leaves of Turkish tobacco plants growing under greenhouse conditions. The individual leaves of these plants were accurately measured and a count made of the number of local lesions which developed on each. The results of one of these experiments are given in Table 4. These results show not only that the leaves of intermediate age and size are the most susceptible to ring-spot infection, but also that the ring-spot symptoms appear in a shorter time on these leaves than on either the very old or the very young ones. At first sight, the results for plant 2 seem to be out of keeping with those of the other plants. This, however, is due to the fact that this plant was a little younger than the others, thereby making the oldest leaf fall in the most susceptible class.

TRANSMISSION OF RING SPOT TO TOMATO BY GRAFTING

Numerous inoculations of tomato with tobacco ring spot by swabbing the leaves have been unsuccessful. Injections of virus-infected juice into the stems also failed to produce infection. To further test the immunity of tomato to the ring-spot virus, scions of tomato were grafted on healthy tobacco plants. After the tomato scions had begun to grow, suckers from the tobacco stock were inoculated with ring spot. The usual symptoms of the ring-spot disease developed on the tobacco suckers. In about three weeks small necrotic spots appeared on some of the tomato leaflets. A few of these spots were in the form of rings and others were in the shape of short necrotic lines or dashes which were arranged end to end as illustrated in Plate 5, C. Large necrotic spots on either side of the midrib were present on a number of the smallest leaflets. (Pl. 5, B.) Several large chlorotic rings were distinguishable, but these were very faint and do not show in the photograph. Healthy tobacco plants inoculated with expressed juice from these tomato leaves developed ring spot, thereby proving that the sap of the tomato carried the virus. At the present time not enough observations have been made to determine whether the virulency of the virus used in this test was affected by its passage through tomato.

SEED TRANSMISSION EXPERIMENTS

Preliminary tests with small lots of seeds sown in the greenhouse suggested that the ring-spot virus might be seed-borne. In these preliminary tests, however, all precautions to prevent secondary infection from other sources were not taken. A test on a larger scale was planned and very carefully carried out. A bed in the greenhouse was thoroughly sterilized with steam by the inverted-pan method. The bed was then divided into small plots (2 by 3 feet) by strips of lumber which were pressed into the soil. The plots were then numbered and sown on September 24, 1929, with seed harvested from ring-spot-infected plants. The majority of the seed had not been harvested longer than a month, and one lot was harvested the same day it was sown.

In a test involving approximately 64,500 tobacco seedlings, not a single one was observed to be infected with ring spot. The plants were not allowed to grow to maturity but it seems reasonable to think that symptoms would have developed in the two months during which the plants were observed had the virus been present.

The result with petunia seed from infected plants was quite different, as has been reported in another paper (*5*). Of the 810 petunia seedlings tested, 160, or 19.8 per cent, developed ring spot. Leaves from these plants when used to inoculate healthy tobacco plants produced typical ring spot. The infected seedlings were severely dwarfed and stunted. (Pl. 6, B.) The first few leaves were mottled in color and streaked with watery green spots that more or less followed the veins. Some of the leaves showed less of the watery green areas and more light-brown streaks, which appeared to be due to a slight necrosis in the tissue. Curling of the leaves in places along the margin was noticeable in some cases, as shown in Plate 6, F. A clearer conception of these symptoms may be secured by comparing the illustrations of the diseased seedlings (pl. 6, B and F) with those of the healthy ones shown in Plate 6, A and E.

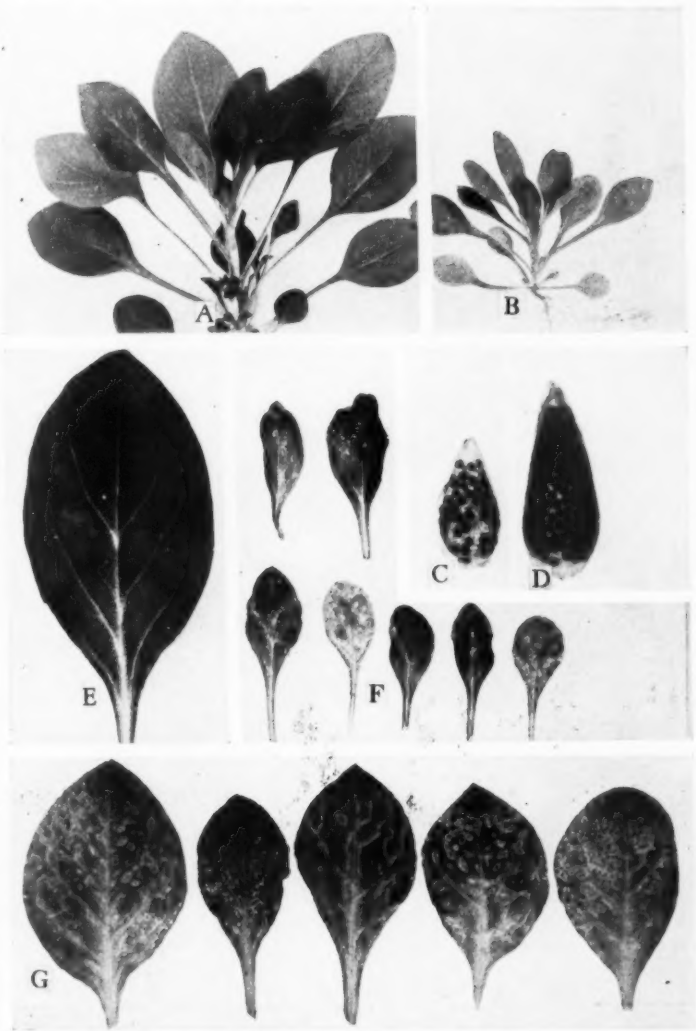
The healthy petunia seedlings were removed from the bed and the infected ones allowed to grow to maturity. For a time these plants remained stunted, but later outgrew the disease and partially masked the symptoms. A number of the leaves continued to show streaks and to develop the chlorotic rings and markings (pl. 6, G) typical of systemic infection. Flowering was profuse, but the seed pods were markedly stunted and bore only a few seeds (pl. 6, C) as compared to the healthy ones (pl. 6, D). Seed saved from these plants was sown in the greenhouse four months after the date of harvest. When the seedlings were about an inch high, notes were taken on ring-spot infection, and about 14 per cent of them were found to be infected.

CROSS-INOCULATION EXPERIMENTS ON TOBACCO AND POTATO

In 1925 Johnson (*8*) reported a study of a disease on tobacco produced by a virus occurring naturally on apparently healthy potato plants. Smith (*13*) later made a more detailed study of this virus. Both writers referred to this disease on tobacco as ring spot. In order to make a comparison of the potato virus disease with the common tobacco ring spot, the writers made cross inoculations on tobacco and potato (*Solanum tuberosum*). Wingard (*15*) had previously reported that he was unable to secure infection on potato with the tobacco ring-spot virus. Priode (*12*) also was unable to secure infection. In a recent bulletin, Johnson of Kentucky (*7*) reported observing Irish Cobbler potatoes naturally infected with tobacco ring spot. However, he did not state whether or not he had inoculated potatoes with the virus from tobacco.

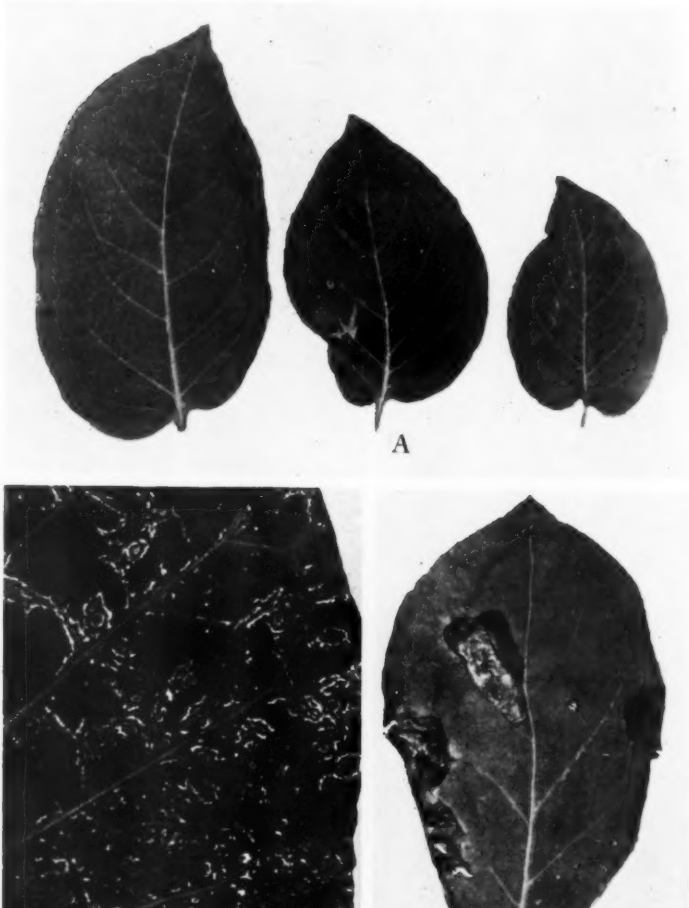
Several small potato plants of an unknown variety growing in the greenhouse were inoculated by rubbing the leaves with cotton saturated with ring-spot-infected tobacco juice. Five days later a number of brown necrotic spots about one-sixteenth to one-eighth of an inch in diameter appeared on the inoculated leaves. Some of the lesions were in the form of rings, but the majority were solid necrotic spots. Plate 7, A, shows the early stage of infection. In two or three weeks the spots enlarged to half an inch in diameter. The old spots were no longer circular in outline but instead conformed more or less to the space between the larger veins. (Pl. 7, C.) No new spots were formed, nor was there any sign that systemic infection had occurred.

Inoculations on tobacco with macerated tissue from leaves showing these brown lesions produced typical ring spot, but at a later date it



TOBACCO RING SPOT ON PETUNIA

A, Healthy seedling; B, seedling of the same age as A infected with ring spot by seed transmission of the virus; C, seed pod from infected plant; D, seed pod from healthy plant; E, leaf from healthy seedling; F, leaves from diseased seedlings; G, symptoms on leaves of mature plants.



A, Tobacco ring-spot infection on leaflets of potato seven days after inoculation; B, symptoms produced on tobacco by the virus occurring naturally on apparently healthy potatoes; C, potato leaflet showing tobacco ring-spot infection 35 days after inoculation. (About natural size)

was observed that some of the symptoms described by Johnson (*8*) were beginning to develop also. There were all indications that two viruses were present. Other inoculations on tobacco with young potato leaves that did not show the brown spots yielded only the Johnson type of ring spot. Inoculations on tobacco with leaves which were taken from the potato plants before they were inoculated with the tobacco ring-spot virus gave the Johnson type of ring spot only. (Pl. 7, B.)

In order to determine whether there was a difference in the susceptibility of potato varieties to ring spot, tubers of five standard varieties were obtained from the agronomy department of the Virginia Polytechnic Institute and planted in the greenhouse along with tubers of the unknown variety previously used. When the vines were about 6 inches high, five plants of each variety were inoculated with ring-spot-infected juice from tobacco. Care was taken to use about the same amount of inoculum on each plant. A total of about 150 leaflets were inoculated on the five plants of each variety.

Table 5 shows the results of these inoculations. Two varieties, Irish Cobbler and Dakota Bake, did not become infected. Early Rose seemed to be the most susceptible, with Bliss Triumph next in susceptibility.

TABLE 5.—*Results of inoculations on standard varieties of potato with tobacco ring-spot virus*

Variety	Approximate number of leaflets inoculated	Leaflets infected	Local lesions produced
		Number	*Number*
Irish Cobbler	150	0	0
Unknown	150	17	34
Early Rose	150	49	125
Bliss Triumph	150	43	63
Dakota Bake	150	0	0
V. P. I. Green Mountain	150	9	14

Even though this experiment was conducted on a small scale, the results obtained indicate a marked difference in varietal susceptibility of potato to ring-spot infection. The results of Wingard and Priode may be explained by the fact that they used only one variety which was probably resistant. Johnson of Kentucky (*7*) stated that the infection which he observed occurring naturally was systemic. In the tests conducted by the writers, with artificial inoculation there was no evidence of systemic infection, and repeated inoculations on tobacco with juice from newly formed potato leaves gave negative results in so far as the symptoms of the tobacco type of ring spot were concerned.

DISCUSSION

The history of tobacco ring spot dates back only a few years, yet this diseases is now known to occur in practically all the commercial tobacco-growing districts of the world. This fact, together with the very broad host range of the disease, makes ring spot a problem worthy of serious investigation.

As in the study of any infectious disease, the problem of foremost importance in the case of ring spot is to locate the source of primary infection and determine how the infective agent is carried over from one crop to the next. So far the only logical theory regarding the carry-over of the tobacco ring-spot virus is that it might overwinter in either the seed or juice of certain weeds and other host plants. It seems very likely that the ring-spot virus might overwinter in certain biennials and perennials as in the case of the viruses of cucumber mosaic, tobacco mosaic, and of several other plant diseases. However, in the one instance of pokeweed infection studied by the writers, the ring-spot virus was not retained overwinter in the rootstocks and the plants grew normally the following year. This does not necessarily mean, however, that the ring-spot virus does not pass the winter in the rootstocks of other perennials, or even in those of pokeweed under different conditions. The important discovery that the ring-spot virus is seed borne in petunia, a nonlegume, suggests that, among the numerous hosts listed by Wingard (*15*), there may be other plants that transmit the virus through their seed. Johnson (*7*) has obtained evidence that the ring-spot virus is seed borne in the case of Turkish tobacco, but from data obtained by the writers it seems that the amount of infection carried over in tobacco seed is too slight to be of very much importance.

There is a possibility that the ring-spot disease may become a serious menace to other crops. There seems to be no end to the list of susceptible hosts. Many of the flowering ornamentals are killed completely or injured so severely as to make them undesirable for commercial use. A number of vegetable crops are especially susceptible to artificial inoculations. The garden bean is usually killed in a short time. Cantaloupes and cucumbers are severely stunted and the fruits are imperfectly formed. The fact, too, that natural infection has been observed on about 40 per cent of the vines in a commercial planting of cantaloupes makes this phase of the problem even more important.

Whether or not the viruses which occur in nature on petunia, sweetclover, yellow ironweed, and squash, and which produce a mild form of ring spot when transferred to tobacco, are the same as the tobacco ring-spot virus remains to be determined. General observations indicate that these viruses are closely related to the tobacco ring-spot virus, yet from a strict comparison of the symptoms produced on tobacco plants, they can not be regarded as identical with it. In view of the fact, however, that certain investigators have obtained evidence which shows that the virulency of a plant-disease virus may be changed when subjected to certain treatments, it does not seem unreasonable to regard these viruses as attenuated forms of the tobacco ring-spot virus. Carsner (*2*), for instance, was able very definitely to attenuate the sugar beet curly-top virus by passing it through either *Chenopodium murali*, *Rumex crispus*, or *Suaeda moquini*. At a later date, Carsner and Lackey (*3*) reported that under certain conditions, not clearly understood, the curly-top virus was also attenuated by passage through resistant beets. In their observations the virus seemed to continue in the mild form even after several transfers on highly susceptible hosts. Lackey (*10*), on the other hand, has recently reported that he was able to restore the attenuated

curly-top virus to its original virulency by passage through *Stellaria media*. Johnson (*9*) found that the tobacco mosaic virus could be attenuated by exposing the tobacco plants soon after inoculation to a temperature of 35°–37° C. for 10 days or more. Repeated transfers on tobacco at a normal temperature did not restore the mosaic virus to its original virulency.

Data obtained by the writers indicate that the petunia virus was in an attenuated form when first transferred to tobacco, but repeated transfers on this host increased its virulency. Merely passing the tobacco ring-spot virus through petunia by artificial inoculation does not affect the virus. Transmission of the virus through the seed of petunia for several generations, however, might change its virulency and bring about this attenuation. As previoulsy stated, work with sweetclover as a host for ring spot has been unsatisfactory. Artificial inoculations of this plant have usually given negative results, yet in some cases it appeared that infection had taken place. In one instance a virus was recovered from artificially inoculated sweetclover and transferred back to tobacco, on which it produced a mild type of ring spot.

Priode (*12*) was unable to secure ring-spot infection from virus-infected tobacco juice after it had been passed through the N grade Berkefeld filter. The writers obtained similar results with juice that had not been purified before filtering; but by removing a large portion of the organic matter by freezing and treating the infected juice with lead acetate and barium acetate solutions, a liquid was obtained which was readily filterable. When this purified juice was used, it was found that the ring-spot virus would pass a Berkefeld filter of the W grade.

The results obtained by the several investigators who have inoculated potato plants with the ring-spot virus have varied. Priode (*12*) and Wingard (*15*) were unable to secure infection on potato. Johnson (*7*) reported natural systemic ring-spot infection on potato; but he failed to state whether or not he made artificial inoculations on this plant. The results obtained by the writers indicate that potato varieties differ widely in their susceptibility to infection by artificial inoculation. These results showed, too, that when infection was obtained on potato it was only local, and apparently never became systemic. The writers are at a loss to explain why the infection did not become systemic in the potato plants used in these tests since it has been reported to occur in systemic form on potato in nature. The use of resistant varieties will, perhaps, explain why Priode and Wingard failed to secure ring-spot infection on potato.

SUMMARY

Ring spot, a virus disease of tobacco, has been reported from a large number of important tobacco-growing districts in the United States, Australia, Sumatra, South Africa, and Nyasaland.

Natural infection has been reported of sweetclover, yellow ironweed, petunia, and squash with viruses which produce ring-spotlike symptoms when transferred to tobacco. These viruses differ from the tobacco ring-spot virus principally in the difference in the severity of the symptoms which they produce on tobacco. It is suggested

that these viruses may possibly be attenuated forms of the tobacco ring-spot virus.

Jimson weed and cantaloupe have been found to be natural hosts of the tobacco ring-spot virus.

The ring-spot virus did not survive the winter in the roots of pokeweed plants.

The thermal death point of the ring-spot virus lies between 60° and 70° C.

Expressed juice from ring-spot-infected tobacco plants stored at a subzero temperature remained infectious for more than 22 months.

The ring-spot virus is very readily inactivated by desiccation.

The ring-spot virus can be precipitated and separated from expressed juice with either alcohol or acetone and recovered in water without any appreciable injury to its infective properties. The virus is filterable through a Berkefeld filter of W grade if the infectious juice is first freed of its suspended solid matter.

The ring-spot virus is infectious in dilutions as great as 1 to 1,000, but only a trace of infection has been obtained with 1 to 10,000 dilutions.

It has been found that under greenhouse conditions the ring-spot virus will persist for more than a year in the juice of tobacco plants which have been propagated by cuttings. The ring-spot symptoms may remain masked during this entire period.

As a result of artificial inoculation, typical ring-spot symptoms developed on detached tobacco leaves and cuttings kept alive in moist chambers. Artificial inoculation also showed tobacco leaves of intermediate age to be more susceptible to ring-spot infection than either the very old or the very young leaves.

Tobacco ring-spot infection was produced on tomato plants by grafting.

Very little evidence has been found in these studies to indicate that the ring-spot virus is seed borne in tobacco, but it was found to be very readily transmitted through the seed of petunia.

Artificial inoculation of certain varieties of potato with the ring-spot virus resulted in local infection on the leaves. Nevertheless, the virus occurring naturally in apparently healthy potato plants is regarded as being entirely different from the tobacco ring-spot virus.

LITERATURE CITED

(1) Butler, E. J.
1928. report on some diseases of tea and tobacco in nyasaland. Nyasaland Dept. Agr. 30 p., illus. Zomba.

(2) Carsner, E.
1925. attenuation of the virus of sugar beet curly-top. Phytopathology 15: [745]–757, illus.

(3) ——— and Lackey, C. F.
1928. further studies on attenuation of the virus of sugar beet curly-top. (Abstract) Phytopathology 18: 951.

(4) Fromme, F. D., and Wingard, S. A.
1922. blackfire or angular-leafspot of tobacco. Va. Agr. Expt. Sta. Tech. Bul. 25, 43 p., illus.

(5) Henderson, R. G.
1931. transmission of tobacco ring spot by seed of petunia. Phytopathology 21: 225–229, illus.

(6) Jochems, S. C. J.
1926. handleiding voor de herkenning en bestrijding van de ziekten van deli-tabak. Meded. Deli Proefsta. Medan ser. 2, no. 43, 39 p., illus.

(7) JOHNSON, E. M.
1930. VIRUS DISEASES OF TOBACCO IN KENTUCKY. Ky. Agr. Expt. Sta. Bul. 306, p. [289]–415, illus.
(8) JOHNSON, J.
1925. TRANSMISSION OF VIRUSES FROM APPARENTLY HEALTHY POTATOES. Wis. Agr. Expt. Sta. Research Bul. 63, 12 p., illus.
(9) ———
1926. THE ATTENUATION OF PLANT VIRUSES AND THE INACTIVATING INFLUENCE OF OXYGEN. Science (n. s.) 64: 210.
(10) LACKEY, C. F.
1931. VIRULENCE OF ATTENUATED CURLY-TOP VIRUS RESTORED BY STELLARIA MEDIA. (Abstract) Phytopathology 21: 123–124.
(11) MOORE, E. S.
1926. DISEASES OF VIRGINIAN TOBACCO IN SOUTH AFRICA. Jour. Dept. Agr. Union So. Africa 12: 428–455, illus.
(12) PRIODE, C. N.
1928. FURTHER STUDIES IN THE RING-SPOT DISEASE OF TOBACCO. Amer. Jour. Bot. 15: 88–93, illus.
(13) SMITH, K. M.
1929. STUDIES ON POTATO VIRUS DISEASES. IV. FURTHER EXPERIMENTS WITH POTATO MOSAIC. Ann. Appl. Biol. 16: 1–33, illus.
(14) VINSON, C. G., and PETRE, A. W.
1929. MOSAIC DISEASE OF TOBACCO. Bot. Gaz. 87: 14–38.
(15) WINGARD, S. A.
1928. HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS. Jour. Agr. Research 37: 127–153, illus.

73318—31——2

THE DENSITY OF STOMATA IN CITRUS LEAVES[1]

By H. S. REED, *Professor of Plant Physiology, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station*, and E. HIRANO, *Professor of Horticulture, Tottori Agricultural College, Tottori, Japan*

INTRODUCTION

The studies to be discussed in this paper were made during the summer of 1929 and were based upon material collected in the various orchards of the Citrus Experiment Station at Riverside, Calif. The leaves were collected from shoots which had grown during the preceding year on the south side of the tree in full sunlight. After the length of the shoots had been measured the leaves were removed in serial order from the base to the apex and their area measured with a planimeter. Small pieces were cut from the median portion of the lamina of each leaf and put into acetic-formalin-alcohol, where they remained until utilized for the study of the stomata. At times during the spring the leaves were kept in a cool, moist chamber and the determinations made on fresh material. A comparison of results obtained by the use of both fresh and killed material showed that no appreciable error was introduced by the use of preserved material.

Tangential sections of the leaf blades were easily cut with a razor and were studied under a microscope equipped with an eyepiece micrometer. It was necessary to treat all sections with a dilute solution of potassium hydroxide, since the younger orange leaves generally contain so much hesperidin that the sections are opaque until the hesperidin is removed. The sections from fresh material were put into 90 per cent alcohol to remove the chlorophyll, then stained with alcoholic Congo red, and finally cleared in dilute potassium hydroxide. The number of stomata in areas of 0.29 sq. mm. were counted and the average of 20 or more areas was recorded. Areas in the vicinity of oil glands, epidermal hairs, and large veins were avoided.

DEVELOPMENT OF THE STOMATA

These studies were made mainly on leaves of Valencia oranges (*Citrus sinensis*, Osbeck) taken from vigorous shoots in the summer of 1929. The stomata develop during the very early stages of growth of the leaf; indeed, fully developed stomata were found in the small imbricated primordial leaves at the tip of the growing shoot. (Fig. 1.) Leaves in this stage also contain many young stomata which can be distinguished by the presence of the septum between the primordial guard cells. Stomata mother cells may also be distinguished from other epidermal cells by their finely granular contents. The adult stomata in these leaves have an average length of 22.6μ and width of 17.2μ.

The long axes of adult stomata in the young leaves are parallel to the long axis of the leaf, since the lateral veins have not reached a conspicuous stage of development. As the leaves grow, the orienta-

[1] Received for publication Feb. 23, 1931; issued August, 1931. Paper No. 243, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

tion of stomata changes and produces an arrangement which will be discussed later. The number of stomata increases as a result of cell divisions, although the more rapid multiplication of intervening cells may result in a reduction of density of the stomata per unit area.

MATURE STOMATA

The size of stomata in a mature leaf is quite variable, the older ones being larger than those formed later in the development of the leaf. Relatively wide areas may occur around large stomata, oil glands, or epidermal hairs in which stomata are lacking. (Fig. 2.) Stomata in species of the genus Citrus are confined to the ventral surface of the leaves. Along the margin of the leaf the stomata have their long axes parallel to the margin; between lateral veins they are parallel to the veins; along the midrib they are parallel to the midrib. (Fig. 3.)

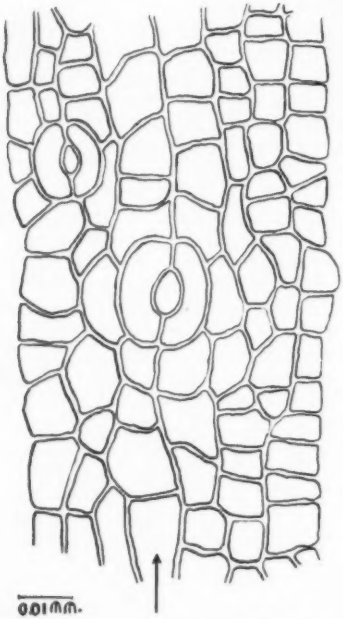


FIGURE 1.—Stomata and epidermal cells in a young primordial leaf of *Citrus limonia* Osbeck (Eureka lemon) the arrow points toward the apex of the leaf

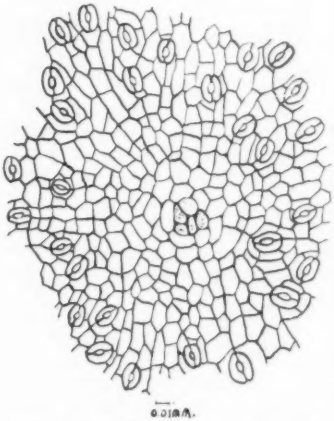


FIGURE 2.—Radial arrangement of stomata around the oil gland of a leaf of *Citrus limonia* Osbeck (Eureka lemon)

The average density of stomata in mature lemon and orange leaves is shown in Figure 4. The figures given were obtained from determinations made on mature leaves of Eureka lemon and Valencia orange taken from shoots which had grown in the preceding year. In each case the average density of stomata was least at the base of the leaf, greatest at the middle, and intermediate at the tip. In some leaves, however, the maximum density of stomata was found in the apical rather than in the middle portion. These observations are not in agreement with those of Bahgat,[2] who found the density less in the middle of the leaf than at the base or the apex. These varia-

[2] BAHGAT, M. M. A STUDY OF THE STRUCTURE AND DISTRIBUTION OF STOMATA IN THE DIFFERENT SPECIES OF CITRUS. [Unpublished thesis, Univ. Calif.] 1923.

tions are probably due to differences in the rate of growth of stomatal mother cells. This phase of the problem needs further study, but there is no apparent difficulty in assuming that the stomatal mother cells should not participate in the greater growth rate of the middle of the leaf.

The production of stomata appears to cease when the young leaves are about one-fourth their final size. Subsequently the density diminishes owing to the continued growth of the intervening cells. After the leaves had reached their final size, no further changes in the density of stomata were observed.

TABLE 1.—*Relation between area of the leaf and density of stomata in growing orange leaves, 1929*

[Areas expressed as square centimeters; stomata expressed as number per square millimeter]

Leaf No.	Item	Area and number of stomata of leaves collected—					Ratios of each measurement to that preceding found on—			
		Aug. 9	Aug. 13	Aug. 19	Sept. 9	Sept. 18	Aug. 13	Aug. 19	Sept. 9	Sept. 18
1	Area	10.5	22.6	61.7	64.7	65.6	2.15	2.73	1.04	1.01
	Stomata	1,085	1,208	434	399	393	.90	2.78	1.09	1.01
2	Area	13.9	25.8	56.1	56.9	58.6	1.86	2.18	1.01	1.03
	Stomata	1,479	943	384	400	394	1.57	2.46	.96	1.01
3	Area	10.5	26.8	65.5	70.0	70.2	2.55	2.44	1.08	1.00
	Stomata	1,078	1,246	507	418	354	.87	2.46	1.21	1.18
4	Area	13.9	33.7	59.2	60.5	61.4	2.43	1.76	1.02	1.01
	Stomata		741	410	381	380		1.81	1.08	1.00
5	Area	6.7	14.5	27.9	29.2	29.5	2.16	1.92	1.04	1.01
	Stomata	1,590	826	428	403	417	1.92	1.93	1.06	.97
6	Area	9.7	20.6	31.7	32.9	33.5	2.12	1.54	1.04	1.02
	Stomata	1,471	890	487	471	445	1.65	1.83	1.03	1.06
7	Area	10.4	19.4	23.9	24.0	24.5	1.86	1.24	1.00	1.02
	Stomata	1,353	694	506	511	483	1.95	1.37	1.01	1.06
8	Area	6.7	17.4	49.0	57.9	59.2	2.60	2.82	1.18	1.02
	Stomata	1,018	1,383	542	441	396	.74	2.55	1.23	1.11
9	Area	10.8	30.6	54.8	57.0	58.4	2.82	1.79	1.04	1.02
	Stomata	1,442	935	491	453	386	1.54	1.90	1.08	1.17
10	Area	6.6	13.2	40.6	45.9	49.5	2.00	3.07	1.13	1.08
	Stomata	899	1,553	499	436	400	.58	3.12	1.14	1.09
11	Area	12.6	30.6	53.4	55.0	56.1	2.44	1.74	1.03	1.02
	Stomata	1,553	813	457	392	395	1.91	1.78	1.16	.99

The evidence upon which these statements rests is presented in Table 1, which gives in some detail the successive measurements of 11 growing leaves of Valencia orange trees. The outline of each leaf was traced on paper and the area measured with a planimeter. A small piece of the lamina was cut from the middle part of the leaf and used for a determination of the number of stomata. Neither operation appeared to have any deleterious effect upon the growth of the leaf. The leaves had an average area of 10.2 sq. cm. when the observations started on August 9, and they then had the maximum density of stomata, except in the case of Nos. 1, 3, 8, and 10, which did not reach the maximum density until August 13. Stomata mother cells were present in all leaves on August 9, but on August 13 there were none except in leaf No. 8. All the leaves examined were on the same shoot and the numerals in the first column of Table 1 give their positions in acropetal sequence.

A survey of the figures in Table 1 shows that there was considerable variability in the size of these leaves at maturity, leaves 5, 6, and 7 being much smaller than the others. The density of stomata in these three leaves was slightly greater than that in the other eight leaves.

In spite of individual differences there is clear evidence that the density of stomata decreased as the area of the leaf increased, after the early stage of stomatal production had passed.

The ratios given in the last four columns of Table 1 indicate that the decrease in stomatal density is closely parallel to the increase in leaf area. The ratios of leaf area show the size of each leaf in relation to its size at the preceding measurement. The ratios of stomatal density are the reciprocals of the ratio between the number on any date and the number on the preceding date. Except on August 13 (when new stomata were being produced on leaves 1, 3, 8, and 10) the ratios show rather close correspondence. It may, therefore, be concluded that the density of stomata in leaves of the orange bears a rather definite relationship to the expansion in area of the leaf during growth. Comparisons between stomatal density of species and genera, therefore, should be based on leaves which have reached their final size.

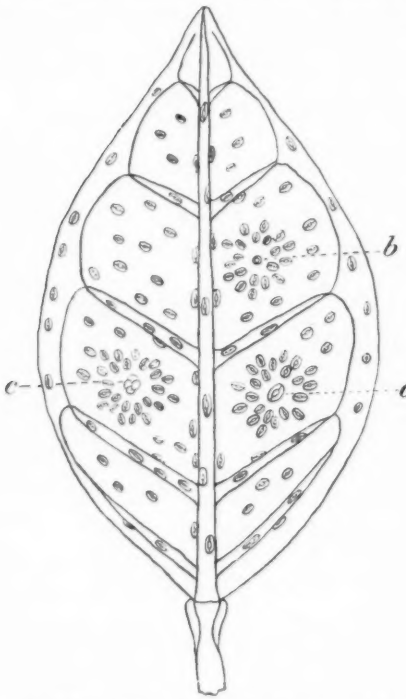


FIGURE 3.—Diagram showing general arrangement of stomata in leaf of *Citrus sinensis* (Valencia orange); *a*, Large stoma; *b*, glandular hair; *c*, oil gland

The problem of the ratio of the size of leaf to stomata density in mature leaves was studied with a much larger sample by biometrical methods, which are better suited to the purpose. The correlation coefficient for size of leaf and density of stomata was determined in a sample of 218 mature lemon leaves. The leaves were taken from 20 shoots which ranged in length from 2 to 162 cm. Leaves were usually selected from each third or fourth node on the shoot, but in the case of the short shoots leaves were taken from each node. Some of the shoots from which these leaves were taken are represented in the graphs shown in Figures 6 to 9, inclusive. Many of the shoots had made two distinct cycles of growth and a few had produced primary laterals.

The size of lemon leaves ranged from 10 to 90 square centimeters and the density of stomata from 380 to 920 per square millimeters. The value of the coefficient of correlation between the two variables is

$$r = -.307 \pm .004$$

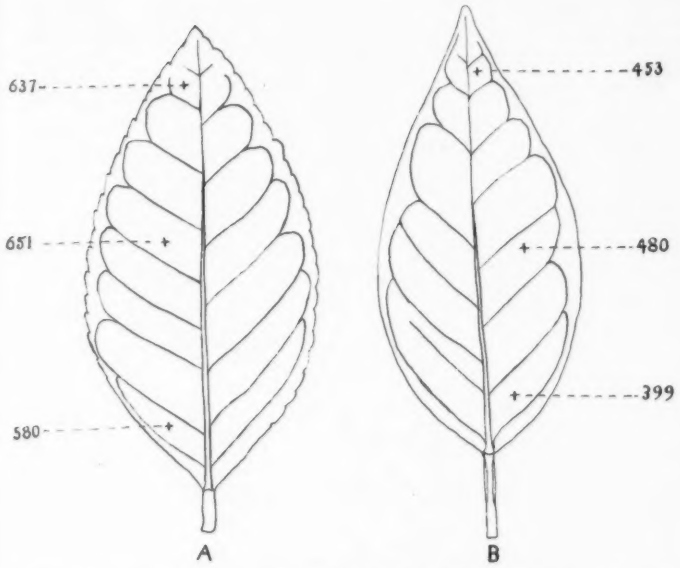


FIGURE 4.—Diagrams showing average density of stomata at base, middle, and apex of citrus leaves: A, *Citrus limonia* (Eureka lemon); B, *C. sinensis* (Valencia orange). Figures indicate the average number of stomata per square millimeter in each area

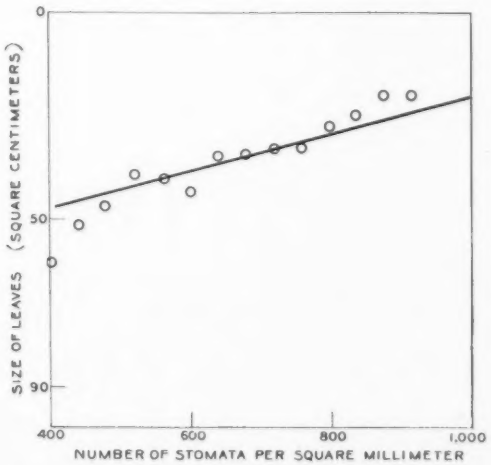


FIGURE 5.—Correlation between size of lemon leaves and density of stomata. Regression line calculated. Small circles represent observed values of y (leaf size)

NUMBER OF STOMATA PER SQUARE MILLIMETER
TOTAL NUMBER OF STOMATA PER LEAF (UNIT OF 10,000)
AREA OF LEAF
LEAF AREA (SQUARE CENTIMETERS)
NUMBER OF STOMATA PER SQUARE MILLIMETER
NODE NO.

FIGURE 6.—Relations between density of stomata, size of leaves, and total number of stomata per leaf to the distribution of leaves on a shoot 163 cm. long selected from *Citrus limonia* (Eureka lemon)

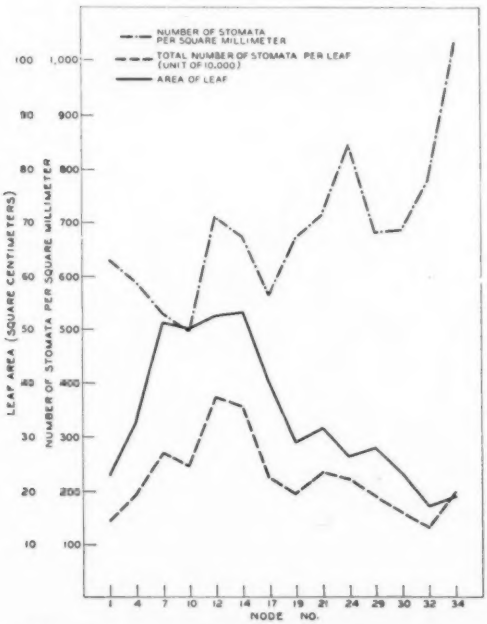

NUMBER OF STOMATA PER SQUARE MILLIMETER
TOTAL NUMBER OF STOMATA PER LEAF (UNIT OF 10,000)
AREA OF LEAF
LEAF AREA (SQUARE CENTIMETERS)
NUMBER OF STOMATA PER SQUARE MILLIMETER
NODE NO.

FIGURE 7.—Relations between density of stomata, size of leaves, and total number of stomata per leaf to the distribution of leaves on a shoot 66 cm. long selected from *Citrus limonia* (Eureka lemon)

The coefficient is negative, as would be expected from the data in Table 1; its magnitude, though not large, indicates a fairly strong correlation between leaf size and stomata density. The mean observed values of leaf size when plotted against the number of stomata per square millimeter (fig. 5) are approximately in a straight line, which agrees closely with the calculated regression line.

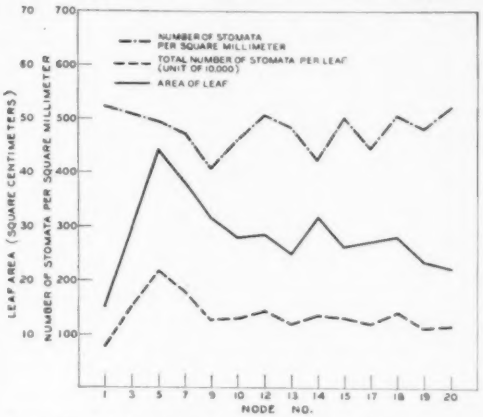

NUMBER OF STOMATA PER SQUARE MILLIMETER
TOTAL NUMBER OF STOMATA PER LEAF (UNIT OF 10,000)
AREA OF LEAF
LEAF AREA (SQUARE CENTIMETERS)
NUMBER OF STOMATA PER SQUARE MILLIMETER
NODE NO.

FIGURE 8.—Relations between density of stomata, size of leaves, and total number of stomata per leaf to the distribution of leaves on a shoot 43 cm. long selected from *Citrus sinensis* (Valencia orange)

RATE OF GROWTH OF THE LAMINA

A series of measurements was made on young Valencia orange leaves after stomata formation had ended, but before growth had ceased, in order to study the growth rate of different parts of the lamina. Sixteen leaves were marked on August 13 with fine parallel lines 6 millimeters apart. One series of lines was parallel to the midrib and the other was perpendicular to it. The lines were made with

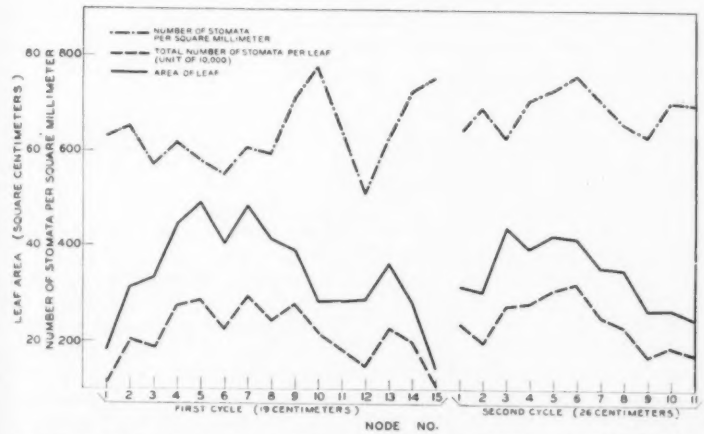

NUMBER OF STOMATA PER SQUARE MILLIMETER
TOTAL NUMBER OF STOMATA PER LEAF (UNIT OF 10,000)
AREA OF LEAF
LEAF AREA (SQUARE CENTIMETERS)
NUMBER OF STOMATA PER SQUARE MILLIMETER
FIRST CYCLE (19 CENTIMETERS)
SECOND CYCLE (26 CENTIMETERS)
NODE NO.

FIGURE 9.—Relation between density of stomata, size of leaves, and total number of stomata per leaf to the distribution of leaves on the first and second cycles of growth of a shoot of *Citrus limonia* (Eureka lemon)

an inked rubber stamp without injury to the leaf tissues. Thirteen days later the leaves were collected and, with the aid of a lens, the distance between the lines was measured. The growth was expressed

as a percentage of the original distance between two or more parallel lines. (Table 2.)

TABLE 2.—*Average growth of various areas of orange leaves in 13 days*

[Growth expressed as percentage of the dimensions marked 13 days earlier]

Direction of measurement	Part measured		Percentage increase
Longitudinal growth	On the midrib	Apex	0
		Middle	13.3
		Base	3.1
	Margin	Left	6.7
		Right	14.5
	Middle of each half blade	Left	12.8
		Right	13.5
Lateral growth	Left half	Apex	6.8
		Near midrib	22.1
		Midway from midrib to margin	17.4
		Near margin	14.9
		Base	14.0
	Right half	Apex	10.3
		Near midrib	17.3
		Midway from midrib to margin	16.6
		Near margin	13.8
		Base	13.8

The figures obtained show a certain amount of variability, but they indicate pretty strongly that growth in expanse was not limited to any portion of the area. Longitudinal growth was at a maximum near the central part of the blade and at a minimum in the apical and basal parts. Lateral growth was likewise greatest in the median area of the leaf, although there was considerable expansion in the apical and basal areas.

Another lot of young orange leaves was marked with fine parallel lines with the rubber stamp on August 16. The formation of stomata was at that time practically completed. The marked areas were measured on August 30 and on September 13. On each of these three dates the stomata on small excised pieces of the leaves were counted. On August 30 the ratio of area to that originally marked was 1.52 and the inverse ratio of stomata was 1.53. On September 13 the ratio of area to the original was 1.62, but the inverse ratio of stomata was still 1.53. These measurements show that the stomatal density in an orange leaf is inversely related to the expansion of the lamina after the process of forming new stomata has ceased.

It should be stated that there is an apparent contradiction between the data presented in Figure 4 and the foregoing statements. The averages of stomatal density in various portions of the citrus leaves seem to be subject to considerable variability. For this there may be two causes: (1) The rate of multiplication of those special cells, the stomata mother cells, and (2) the rate of growth of the epidermal cells. One need not assume that the two grow at the same rate. As has already been said, there is no apparent reason for assuming that the stomatal mother cells do not multiply at a more rapid rate than the surrounding epidermal cells.

POSITION OF LEAVES ON THE SHOOT IN RELATION TO SIZE AND STOMATAL DENSITY

The next studies on citrus leaves were undertaken for the purpose of learning whether the spatial sequences on the shoot showed any definite relationship to the size of leaves or to the density of the stomata. Zalenski's law [3] predicates that the morphological differentiation of a leaf is a function of its position on the shoot. Sorauer [4] observed that the number of stomata per unit of surface increased and the size of the stomata decreased, the greater the distance of the leaf from the surface of the soil. Reed [5] showed that the relationship between apricot shoots and their primary laterals is very definite and supports the idea that the development of the primary laterals is an expression of a definite physiological function of growth.

The citrus leaves afforded a good opportunity to extend the biometrical investigations of previous workers. The size of leaf and the density of stomata were determined for 218 mature leaves of Eureka lemon and 22 of Valencia orange. The lemon leaves were taken in acropetal order from 20 shoots which ranged from 2 to 162 cm. in length.

Leaves from each shoot were generally selected from each third or fourth node, but in the case of the short shoots leaves were taken from each node. Many of the shoots had made two distinct cycles of growth and a few had produced primary laterals.

Typical measurements obtained from this material are represented by the graphs in Figures 6, 7, 8, and 9.

It will be seen that the leaves at the base of the shoot are usually small and that the size increases to a maximum in a region about one-fourth or one-third of the distance from the base to the apex of the shoot, then decreases rather regularly to the apex, where small leaves are found. The smallest leaves may be only one-seventh the size of the largest leaves on the same shoot. This tendency to differentiation is not so clearly manifest in short shoots as in long ones.

The graphs show that the density of stomata on the leaves had a certain inverse correspondence to the size of the leaves. Leaves near the apex of the shoot had the greatest, and the large leaves one-third of the distance from base to apex had the smallest density of stomata. It should be remarked, however, that the differences in density are not at all proportional to the differences in the size of the leaves.

The shoot represented in Figure 9 made two distinct cycles of growth during the season; the first cycle was 19 and the second 26 cm. long. The leaves on the first cycle of growth ranged in area from 15 to 50 sq. cm.; those on the second cycle were somewhat smaller and more uniform in size. The graphs show that in both cycles the maximum size of leaves was attained at a place on the shoot about one-third to one-fourth the distance from base to apex of the cycle of growth concerned, and that the size diminished quite regularly from that point to the apex.

---

[3] ZALENSKI, W. VON. UEBER DIE AUSBILDUNG DER NERVATION BEI VERSCHIEDENEN PFLANZEN. Ber. Deut. Bot. Gesell. 20: 433-440. 1902.
——— [MATERIALS FOR THE STUDY OF THE QUANTITATIVE ANATOMY OF DIFFERENT LEAVES OF THE SAME PLANT.] Izv. Politek. Inst. Kiev (Mem. Polytech. Inst. Kiev) 4: 1-203. 1904. [In Russian.]
[4] SORAUER, P. UEBER DEN EINFLUSS DER LUFTFEUCHTIGKEIT. Bot. Ztg. 36: [1]-13, [17]-25. 1878.
[5] REED, H. S. GROWTH AND DIFFERENTIATION IN APRICOT TREES. Calif. Univ. Pubs., Agr. Sci. 5 (1) 1-55, illus. 1924.

In all the graphs the total number of stomata per leaf is also shown. It is apparent that the number corresponds rather closely to the area of the leaves.

Taken in a general way the data given in the graphs support the idea that there is a definite relation between morphological differentiation and spatial position. The leaves near the base of the long shoots were usually small in size, but the density of stomata on them was not as high as that of the small leaves near the apex of the shoot. There may be many reasons for this difference, but one thinks first of the effect of light. The basal leaves are generally more shaded during the time of their development than the apical leaves. Bahgat [6] observed that shaded orange leaves grown under a thin cloth tent had fewer stomata than leaves from unshaded trees. Gourley and Nightingale [7] and Maximov [8] have observed similar effects with other plants. Shading has the general effect of reducing the density of stomata and of increasing the surface area of epidermal cells. The leaves on the shorter shoots showed less tendency to conform to Zalenski's law. The leaves on the two orange shoots showed no systematic differences in respect to size or number of stomata per unit area.

FACTORS WHICH AFFECT THE SIZE OR DENSITY OF STOMATA

CROWDING

Four mature leaves were selected from the lemon shoot represented in Figure 7. The leaves were from nodes 10, 17, 24, and 34 and might be regarded as typical of the systematic variation in stomatal density described above. Table 3 shows the measurements of average size and density of stomata on leaves which ranged from large to small. The small leaves at the apex of the shoot had many more stomata than those near the base, yet the difference in size as expressed in the table has very doubtful significance.

TABLE 3.—*Relation between size of leaf and density of stomata on lemon leaves from different nodes*

Item	Enumeration or measurements recorded for leaf No. (in order from base of the shoot)—			
	10	17	24	34 [b]
Area of leaf, square centimeter	50.05	40.30	26.59	11.57
Stomata per square millimeter, number	496	563	846	1,033
Length of stomata,[a] μ	19.8	19.3	18.0	17.8

[a] Average of 25 stomata which did not contain the big central stomata of centrifugal arrangement.
[b] Top leaf.

UNEQUAL GROWTH OF LAMINA ON OPPOSITE SIDES OF MIDRIB

Three types of abnormal orange leaves were studied (1) lamina on one side of midrib less than half the area of the other side; (2) one side of lamina showing "crinkle"; (3) one side of lamina swollen. Table 4

[6] BAHGAT, M. M. Op. cit. (See footnote 2.)
[7] GOURLEY, J. H., and NIGHTINGALE, G. T. THE EFFECTS OF SHADING SOME HORTICULTURAL PLANTS. A PRELIMINARY REPORT. N. H. Agr. Expt. Sta. Tech. Bul. 18, 22 p., illus.
[8] MAXIMOV, N. A. THE PLANT IN RELATION TO WATER; A STUDY OF THE PHYSIOLOGICAL BASIS OF DROUGHT RESISTANCE. Authorized English translation, edited with notes by R. H. Yapp, p. 346-347. London. 1929.

gives a comparison of the number of stomata in both parts of the leaves, and shows that there was no significant difference to be ascribed to the unequal growth of the lamina. This result is out of harmony with those previously reported on the relation between the growth of the lamina and the density of stomata on it. It seems logical to conclude, either that the deformities arose very early, or that the specific factors which accelerated the growth of the laminae also accelerated the formation of stomata.

TABLE 4.—*Average density of stomata in normal and deformed parts of orange leaves*

Variety of orange	Area of leaf	Part studied	Areas studied	Stomata per square millimeter
	Sq. cm.		*Number*	*Number*
Lue Gim Gong	39.0	Narrow side of lamina (11.18 sq. cm.)	20	419
		Wide side of lamina (27.82 sq. cm.)	20	420
Valencia	148.8	Normal side of lamina	30	273
		Crinkled side of lamina	30	295
Do	133.3	Normal side of lamina	30	380
		Swollen side of lamina	30	403

PRODUCTION OF FLOWERS AND FRUIT

In view of certain well-defined physiological differences correlated with vegetative and fruiting shoots, it seemed desirable to ascertain the density of stomata in the two types. For this study five cognate shoots on a branch of a Valencia orange tree were selected. At the time of selection (August 7) all leaves were mature. Two of the shoots were without flowers or fruit, two had fruits, and one had produced flowers but no fruit had set. There were therefore two vegetative and three fruiting shoots. The other characters are shown in Table 5.

TABLE 5.—*Effect of fruiting or blossoming on the density of stomata on Valencia orange leaves, August 7, 1929*

Shoot [a]	Length of shoot	Condition of shoot	Measurement [b]	Enumeration or measurement recorded for leaf No. (in order from base of shoot)—											
				1	2	3	4	5	6	7	8	9	10	11	Average
	Cm.														
A	10	No flowering	Number of stomata per square millimeter.	665	685	595	593	610	599	595	636	555	.....	.....	615
			Size of leaf in square centimeters.	107	62	104	117	101	84	49	69	55	.....	.....	83
B	8	A young fruit weighing 19 gm. at the top of shoot.	Number of stomata per square millimeter.	633	605	595	669	565	565	604	.....	.....	.....	.....	605
			Size of leaf in square centimeters.	58	55	93	88	106	97	79	(c)	.....	.....	.....	82
C	12	Flowered, but no fruit at the top of shoot and at axils of leaves of 3, 4, 5, and 6.	Number of stomata per square millimeter.	530	623	675	659	621	751	623	618	565	570	715	632
			Size of leaf in square centimeters.	124	148	[d] 126	[d] 135	[d] 136	[d] 124	104	80	61	45	39	102
D	9	No flowering	Number of stomata per square millimeter.	693	692	677	537	624	621	612	577	602	630	.....	627
			Size of leaf in square centimeters.	32	65	60	87	110	99	110	58	34	58	.....	71
E	8	A young fruit weighing 12 gm. at the top of shoot.	Number of stomata per square millimeter.	630	611	600	692	449	554	595	658	646	.....	.....	604
			Size of leaf in square centimeters.	116	191	174	44	84	80	63	31	31	(c)	.....	90

[a] A shoot is the nearest to the base of mother shoot, E shoot is the farthest to the base of mother shoot.
[b] Number of stomata is the average of 10 counts on each leaf.
[c] Location of young fruit.
[d] Location of blossoms.

In general the size of leaves and the average density of stomata were remarkably uniform in these five shoots. The production of flowers and fruit seems to have had no appreciable effect on the production of stomata even on leaves adjacent to fruits. This may be due to the fact that the leaves develop and the number of stomata is constant before the fruits have reached a stage where any physiological effect can be exerted upon the leaves.

LIGHT

Since light is the source of energy for plant activity it may be expected that the intensity of light will effect the density of the stomata as a result of cell division and growth. The results of previous work show a reduction in the density of stomata in weak light. Two species were used in this study; *Citrus limonia* (Eureka lemon) and *Chalcas exotica*. Two sets of 10 lemon leaves, each of similar size, were taken from 10 spring shoots which grew in full sunlight and an equal number from shoots which grew in shade inside the tree. With Chalcas, the terminal leaflets were taken from different trees, one of which grew in full sunshine, the other under lath where the intensity of light was greatly reduced. Twenty areas were counted on each leaf. The comparative density of stomata is shown in Table 6.

TABLE 6.—*Influence of light*[a] *on the density of stomata in leaves of citrus fruits*

Species of fruit and lighting	Average area of leaf	Leaves	Average number of stomata in 1 square millimeter	Approximate number of stomata per leaf
Citrus limonia (Eureka lemon):	*Sq. cm.*	*Number*		*Thousands*
In sunshine	37.2	10	636	2,366
In shade inside the same tree	37.8	10	322	1,217
Chalcas exotica:				
In sunshine	10.7	10	501	536
Under lath	10.5	10	341	358

[a] Intensity of light was not measured.

Shading reduced the density of stomata in both Eureka lemon and Chalcas. Accordingly, the total number of stomata on the leaf was reduced to approximately the same degree because the study was made with leaves of similar size. The results may have been modified by other factors, such as lower temperature, slightly higher relative humidity, etc., which accompanied the shading; therefore, this result may be said to be the combined result of these factors, although the main effect was caused by difference of light.

CULTURE MEDIA

Observations were made on the stomata of young lemon trees growing in water cultures, sand cultures, and soil cultures. The material was furnished by A. R. C. Haas, of the department of plant physiology.

The plants in water culture were grown in a greenhouse under a glass roof which had been whitewashed and lined with paper, so that the intensity of light was greatly diminished. The plants which grew in pots containing quartz sand or soil were under lath in the open

field where the light was reduced but not so great as in the glass house. These three plants received similar nutrient solutions and the moisture content of the sand and soil cultures was kept near the optimum. All plants made normal growth. Mature leaves taken from each tree were used for the examination. The results are shown in Table 7.

TABLE 7.—*Density of stomata in Eureka lemon leaves when plants were grown in different culture media*

Conditions of growth	Average area of leaf	Leaves	Average number of stomata per square millimeter	Approximate number of stomata per leaf
	Sq. cm.	*Number*		*Thousands*
Water culture, in greenhouse	36.7	6	503	1,846
Sand culture, under lath	36.4	6	650	2,366
Soil culture, under lath	36.5	6	674	2,460

The density of stomata in leaves of the small tree in water culture was about 75 per cent of that of the tree in soil culture. The trees in sand and soil had practically the same density of stomata; this similarity may be due to the fact that the moisture in both was kept almost at the optimum.

SUMMARY

The stomata of citrus leaves are produced during the early stages of development of the leaf and are confined to the ventral surface of the lamina.

The density of the stomata varies in different parts of the leaf and in different species of Citrus. Stomata are more abundant in lemon than in orange leaves. The density per unit area is greatest in very young leaves at the end of the period of stomatal production. Following this period there is a certain relationship between the growth of the leaf and the density of stomata. The coefficient of correlation between the average size of mature lemon leaves and the density of stomata was $-.307 \pm .004$.

The maximum growth in area of orange leaves is near the center of the lamina, the minimum near the apex.

The density of stomata on the leaves of lemon shoots shows a certain relationship to the position of the leaves on long shoots, but the relationship is not well marked on short shoots.

The size of stomata seems to be only slightly influenced by the number per unit area.

The density of stomata is reduced when the intensity of light is reduced.

PRELIMINARY STUDIES OF THE ENZYMES OF GIBBERELLA SAUBINETII[1]

By GEORGE SPITZER, *Associate in Dairy Chemistry, Department of Dairy Husbandry,* and MAURICE M. DIEHM, *Assistant in Agronomy, Department of Agronomy, Purdue University Agricultural Experiment Station*[2]

INTRODUCTION

Gibberella saubinetii (Mont.) Sacc., the fungus causing the commonly known scab disease of maize, oats, wheat, barley, and a few other cereals, has engaged the attention of scientists for some time as a disease of some of the world's most important economic crops. Its physiological properties and characteristics are fairly well established among botanists, but the biochemical properties of the organism are not so well known. Studies on the enzymic activity of the fungus were undertaken in order to give more information concerning some of the chemical changes that occur within the affected host tissues, and possibly to throw some light on other enzymic properties which have hitherto been unknown.

REVIEW OF LITERATURE

It is known from investigations that cereals attacked by this fungus are toxic when eaten by animals. Naumov (*8*)[3] submitted evidence that wheat grains affected by scab acquire toxic properties that are subsequently communicated to the flour and bread. The toxin produced, supposed to be a nitrogenous glucoside, when injected into frogs resulted in death in three or four hours. Naumov's work is corroborated in part by Bennett (*1*). Mundkur and Cochran (*7*) report that hogs fed on an exclusive diet of infected barley developed nausea for food and starved rather than eat it. Guinea pigs also rejected such an exclusive barley diet. Mature chickens, however, when fed with scabby barley showed no symptoms of poisoning. Dickson and others (*2*) found that the water extract from badly scabbed barley produced acute vomiting in pigs. The extract when freed from proteins, polysaccharides, and those nitrogenous substances precipitable with tannic acid was more acute as an emetic for pigs than the original crude extract. The active substances seemingly are associated with the fractions containing glucosides or basic nitrogen compounds. Roche and his coworkers (*9*) have found that pigs, horses, and dogs, as well as man, are very sensitive to the accumulated products in the affected grains and will not tolerate even low percentages of badly scabbed kernels. However, the farm utilization of scabby barley may be economical because it can be fed to cattle, sheep, and poultry without ill effects.

[1] Received for publication Feb. 26, 1931; issued August, 1931.
[2] The writers wish to acknowledge the cooperation of the botany department, particularly Dr. E. B. Mains.
[3] Reference is made by number (italic) to Literature Cited, p. 229.

GROWTH OF THE SCAB FUNGUS IN THE LABORATORY

The liquid medium best suited for the cultivation of the scab fungus consisted of a 4 per cent malt extract with the addition of 0.2 per cent nitrate of soda. Various other liquid media of both organic and inorganic natures were tried but did not prove satisfactory for the work. Among them were the media used by Ellenberger (*4*) and Leonian (*6*). The medium used was about at the neutral point at the time of inoculation and became slightly acid (usually about pH 5.5 to 5.8) as the growth of the fungus progressed.

The malt-extract medium was prepared by finely grinding malted barley and extracting with distilled water for an hour or longer. After the solution had stood for the required length of time it was filtered to remove the undissolved material. To the filtrate was then added 0.2 per cent nitrate of soda, and the medium was brought up to volume in a volumetric flask. After sterilizing the medium, it was filtered to remove the flocculent precipitate. Resterilizing for 25 to 30 minutes at 15 pounds pressure left the medium perfectly clear with no precipitate.

This resulting medium was inoculated with mycelial growth from test-tube cultures on potato-dextrose agar. The maximum growth was obtained at a temperature of 25° C. on about 1 cm. depth of medium in Erlenmeyer flasks.

On the tenth day, at which time the maximum growth had been produced and showed the pinkish-red coloration, the matted mass on the surface of the medium was removed and dehydrated with acetone and ether according to the method of Dox (*3*). The desiccated mass was dried at room temperature until the odor of ether was no longer perceptible, and the dry mass was ground thoroughly in a mortar. The resulting pinkish-red product was used in making the enzyme studies. It can be kept several months in a dark place and in an air-tight bottle.

COMPOSITION OF ENZYME MATERIAL

The enzyme material contains, besides the enzymes, all the cell constituents that are insoluble in acetone and ether. The acetone from the first dehydration has a reddish-pink color. The residue left after distilling off the acetone resembles lipoids. The ground, extracted enzyme material contains 0.1115 per cent ash and 6.16 per cent total nitrogen. The aqueous extract gives positive tests for the biuret and Hopkins-Cole reactions.

METHODS

In the experiments about to be described the source of enzyme was the prepared powder, thoroughly ground in a mortar to which had been added a few drops of water, glycerin, and a small quantity of sand. The groundmass was transferred with distilled water to the volumetric flasks containing the appropriate substrate, and the contents of the flask were brought up to volume with distilled water to give the correct percentage concentrations of both the enzyme and substrate. The advantage of this method is that a preparation of uniform activity can be used in a whole series of experiments.

The digestions were carried out at room temperature (about 25° C.). The surface of each digestion mixture was kept covered with toluol which served as an antiseptic. All the digestion tubes and flasks were tightly stoppered to prevent any change in volume by evaporation of the solvent and any loss of toluol. Aliquot portions taken for analysis were carefully measured with a pipette. The time of digestion varied in the different experiments but is stated in each case. Control experiments were made from each digestion mixture, and aliquot samples were taken in each case immediately after the solutions were made up to the correct volume and before any appreciable enzymatic action could take place.

The scab organism was tested for its activity on the following substrates: Amygdalin, salicin, sucrose, hydrogen peroxide, olive oil, casein, gelatin, urea, starch, and cellulose.

EXPERIMENTAL DATA

GLUCOSIDASE

The enzyme material was tested for its glucoside-hydrolyzing power by using as substrates amygdalin and salicin. For each test 1 gm. of the glucoside was dissolved in about 60 c. c. of distilled water in a 100 c. c. volumetric flask. To this was added an enzyme mixture representing 0.1 gm. of dry material. The solution was then brought up to volume in the flask, thus giving concentrations of substrate and enzyme material of 1 and 0.1 per cent, respectively. The digestion mixtures were tested at various times with Fehling's solution to determine the amount of dextrose present, which had been formed by the hydrolysis of both amygdalin and salicin. The results are given in Table 1.

TABLE 1.—*Hydrolysis of amygdalin and salicin by the enzyme material prepared from Gibberella saubinetii*

HYDROLYSIS OF AMYGDALIN			HYDROLYSIS OF SALICIN		
Time	Quantity hydrolyzed	*K*	Time	Quantity hydrolyzed	*K*
	Per cent			*Per cent*	
23 hours	10.79	0.0049654	16 hours	10.05	0.006621
30 hours	13.63	.0048854	24 hours	15.07	.006807
48 hours	18.47	.0042548	48 hours	22.86	.005410
55 hours	20.04	.0040671	65 hours	28.06	.005146

Both of these glucosides were quite readily hydrolyzed by the enzyme material. No attempts were made to determine the time necessary for the hydrolysis to stop or go to completion. The reaction constants

$$K = 2.303 \frac{1}{t} \operatorname{Log}_{10} \frac{a}{a-x}$$

are fairly uniform in each experiment, especially in that of the hydrolysis of amygdalin. As shown by the reaction constants, salicin was hydrolyzed more rapidly than amygdalin. Generally speaking, the constants decreased slightly toward the end of each experiment.

INVERTASE

Four grams of cane sugar was dissolved in about 60 c. c. of distilled water in a 100 c. c. volumetric flask. To this was added 0.1 gm. of enzyme material, as in the previous experiment. The solution was then brought up to a volume to give a concentration of 4 per cent sucrose and 0.1 per cent enzyme material. The amount of hydrolysis was determined at various times by making reduction tests with Fehling's solution. The results of the experiment are given in Table 2.

TABLE 2.—*Hydrolysis of sucrose by the enzyme material prepared from Gibberella saubinetii*

Time	Quantity hydrolyzed	K	Time	Quantity hydrolyzed	K
	Per cent			*Per cent*	
4 hours	12.68	0.033856	24 hours	57.70	0.035856
10 hours	28.10	.032952	30 hours	67.50	.037472
22 hours	53.35	.035303	34 hours	68.75	.034217

The table shows that the material contains a very active enzyme capable of hydrolyzing sucrose. The hydrolysis progressed rapidly and at a fairly definite rate, as shown by the velocity constants. Even after 34 hours and 68 per cent hydrolysis of the sucrose, the velocity of the reaction had not decreased to a point below the average rate of the reaction.

CATALASE

The reaction of the enzyme catalase was determined by measuring the volume of oxygen gas liberated from hydrogen peroxide (*10*). One-tenth gram of the enzyme material was added to 25 c. c. of 3.6 per cent hydrogen peroxide, and the gas evolved was collected and measured over water in a gas burette. Table 3 shows the activity of the enzyme material.

TABLE 3.—*Catalase activity of the enzyme material from Gibberella saubinetii as determined by the liberation of oxygen gas from hydrogen peroxide*

Time	Oxygen gas liberated	$\frac{x}{t}$	$K=\frac{x}{\sqrt{t}}$
	c. c.		
2 minutes	3.8	1.90	2.67
4 minutes	4.8	1.20	2.40
6 minutes	5.2	.87	2.12
8 minutes	5.4	.67	1.92

The velocity of the reaction decreases as the time increases. This reaction does not follow the monomolecular law but approaches Schütz's rule. Considering the fact that only 0.1 gm. of enzyme material was used, Table 3 shows that the enzyme catalase in the material is relatively active. After eight minutes, however, the reaction stopped, since no more oxygen was evolved.

LIPASE

The enzyme material was tested for its lipolytic activity by using an emulsion of olive oil at pH 4.7, which is suggested by Willstätter and Waldschmidt-Leitz (*5, p. 16*). Fifteen cubic centimeters of the emulsion were placed in each of four test tubes, and to each tube was added a quantity of a solution representing 0.1 gm. of dry enzyme material. At the time indicated each tube was titrated according to the method of Willstätter and Waldschmidt-Leitz (*11*) to determine the acidity produced by the hydrolysis of the oil. The percentage of oil hydrolyzed is shown in Table 4.

TABLE 4.—*Hydrolysis of olive oil by the enzyme material prepared from Gibberella saubinetii*

Test-tube No.	Time	Oil hydrolyzed	$K=\frac{x}{\sqrt{t}}$
	Hours	*Per cent*	
1	0	0	
2	24	1.48	0.30
3	48	2.36	.34
4	72	2.95	.35

There was some hydrolysis of the oil, even though the reaction was not very strong. It must be noted, however, that since the oil was partially hydrolyzed, the reaction cannot be disregarded. The reaction diminished as the time progressed, with considerable retardation on the third day. The reaction does not follow the monomolecular law, but when Schütz's rule is applied it is found that the transformation is proportional to the square root of the time.

PROTEASES

The proteolytic activity of the enzyme material was determined by using both casein and gelatin as substrates. The casein solution was prepared by suspending 4 gm. of casein in 160 c. c. distilled water, adding 16 c. c. 0.2N NaOH and then heating to about 40° C. Then it was heated to 95° C. to inactivate the enzymes and bacteria present in the solution. This solution is diluted to approximately 200 c. c. and adjusted to pH 9. Enzyme material corresponding to 0.2 gm. of dry material was added, and the solution was made up to 200 c. c. with distilled water. The same procedure was repeated for the tests at pH 6 and at pH 3. In the preparation of the gelatin solution 2 gm. were dissolved in about 50 c. c. water, diluted to approximately 100 c. c. and sterilized. To this, NaOH solution was added to bring the solution to pH 6, and enzyme material added corresponding to 0.1 gm. of dry material and the volume made up to 100 c. c. The hydrolysis of these proteins was measured by determining the increase of both the monoamino acids and peptones by precipitating with 5 per cent phosphotungstic acid and saturated zinc sulphate solutions, respectively.

The phosphotungstic acid does not precipitate the monoamino acids in solution, but it does precipitate all other proteins, including the diamino acids. The saturated zinc sulphate solution precipitates all the proteins except the amino acids, peptids, and peptones. By determining the total nitrogen left in the filtrates after precipitation,

the amount of nitrogen in each group and the amount of nitrogen not precipitated by phosphotungstic acid and saturated zinc sulphate solution may be calculated. The amount of nitrogen not precipitated by zinc sulphate minus the amount not precipitated by phosphotungstic acid gives the amount of nitrogen in the form of diamino acids, peptids, and peptones, which are frequently designated collectively as peptones.

TABLE 5.—*Hydrolysis of casein and gelatin by the enzyme material prepared from Gibberella saubinetii*

pH	Time	N not precipitated by phosphotungstic acid	N not precipitated by zinc sulphate	N as peptones
	HYDROLYSIS OF CASEIN			
	Hours	*Per cent*	*Per cent*	*Per cent*
9	0	2.63	8.74	6.11
	24	4.72	12.93	8.21
	48	6.82	15.03	8.21
	72	6.82	17.14	10.32
6	0	2.63	8.74	6.11
	24	6.13	12.26	5.13
	48	9.63	15.74	6.11
	72	12.25	19.25	7.00
3	(a)			
	HYDROLYSIS OF GELATIN			
6	0	2.62	4.50	1.88
	48	6.82	21.00	14.18

[a] No action.

Table 5 shows that there was considerable hydrolysis at both pH 9 and pH 6. There was more hydrolysis of the casein at pH 6 than at pH 9 and no hydrolysis at pH 3. This would indicate that the enzymes in this organism are of the tryptic and ereptic types and possibly a pepsinase, which the results at pH 6 suggest.

The gelatin was quite rapidly hydrolyzed at pH 6, and the enzyme material was more active in the formation of peptones from gelatin than from casein at pH 6.

UREASE, AMYLASE, AND CELLULASE

Tests were made for the action of urease, amylase, and cellulase by using urea, starch, and cellulose, respectively, as substrates, but no action was obtained from any of them. The fact that these enzymes were not found to be active in the dry enzyme material does not prove that they were not present in the living organism.

SUMMARY

In the study of the organism *Gibberella saubinetii* a medium was used that consisted of 4 per cent malt extract and 0.2 per cent nitrate of soda, on which the scab fungus grew in a manner to meet the requirements for the enzyme studies of the dehydrated material.

The hydrolysis of the glucosides amygdalin and salicin by the enzyme material showed a decided activity, indicating the presence of glucosidase.

The hydrolysis of sucrose by the enzyme material was much more rapid than that of the glucosides by glucosidase, as shown by the respective velocity constants, and the presence of invertase was indicated.

The enzyme material showed a decided catalase reaction.

The action on olive oil was weak but sufficient to prove the presence of lipase.

The proteins casein and gelatin were used as substrates to study the proteolytic properties of the extract. Casein was hydrolyzed readily at pH 9 and pH 6, but not at pH 3, indicating the presence of proteolytic enzymes, especially trypsin and erepsin. The results for gelatin are in general agreement with those for casein.

No positive results were obtained for urease, amylase, and cellulase.

LITERATURE CITED

(1) Bennett, F. T.
1928. on two species of fusarium, f. culmorum (w. g. sm.) sacc. and f. avenaceum (fries) sacc. as parasites of cereals. Ann. Appl. Biol. 15: 213–244, illus.

(2) Dickson, A. D., Link, K. P., Roche, B. H., and Dickson, J. G.
1930. report on the emetic substances in gibberella-infected barley. (Abstract) Phytopathology 20: 132.

(3) Dox, A. W.
1909. the intracellular enzymes of lower fungi, especially those of penicillium camemberti. Jour. Biol. Chem. 6: 461–467.

(4) Ellenberger, W.
1915. zur frage der cellulose verdauung. nach versuchen a. scheunert, w. grimmer und a. hopffe. Hoppe-Seyler's Ztschr. Physiol. Chem. 96: [236]–254.

(5) Euler, H. von
1928. chemie der enzyme. teil 2, abschnitt 1, die hydrolysierendenenzyme der ester, kohlenhydrate und glukoside . . . Aufl. 3, t. 2. abschnitt 1, 473 p., illus. München.

(6) Leonian, L. H.
1929. studies on the variability and dissociations in the genus fusarium. Phytopathology 19: 753–868, illus.

(7) Mundkur, B. B., and Cochran, R. L.
1930. some feeding tests with scabby barley. (Abstract) Phytopathology 20: 132.

(8) Naumov, N. A.
1917. intoxicating bread. Trudy Biur. Mik. i. Fitopat. no. 12, 216 p., illus. Petrograd. [Reviewed by M. Shapovalov in Phytopathology 7: [384]–386. 1917.]

(9) Roche, B. H., Bohstedt, G., and Dickson, J. G.
1930. feeding scab-infected barley. (Abstract) Phytopathology 20: 132.

(10) Smolik, L.
1925. hydrogen-peroxide catalase of marsh soils. Internatl. Soc. Soil Sci. Proc. 1: 105–119, illus.

(11) Willstätter, R., and Waldschmidt-Leitz, E.
1921. alkalimetrische bestimmung von aminosäuren und peptiden. Ber. Deut. Chem. Gesell. 54: 2988–2993.

THE INFLUENCE OF NITROGEN, PHOSPHORIC ACID, AND POTASH ON THE NUMBER, SHAPE, AND WEIGHT OF POTATO TUBERS [1]

By WM. H. MARTIN, *Plant Pathologist, New Jersey Agricultural Experiment Station;* B. E. BROWN, *Senior Biochemist, Office of Soil Fertility Investigations, Bureau of Chemistry and Soils, United States Department of Agriculture;* and H. B. SPRAGUE, *Agronomist, New Jersey Agricultural Experiment Station* [2]

INTRODUCTION

As a result of numerous investigations, marked changes have taken place in recent years in methods of fertilizing potatoes. In many sections where potatoes are intensively grown the tendency has been to apply larger amounts of fertilizer. There has also been a growing tendency to derive larger amounts of nitrogen from inorganic rather than from organic nitrogen materials. More recently there has been a marked trend in the use of concentrated fertilizer mixtures. These changes have resulted in a more economical use of fertilizers and in general have been responsible for decreased production costs.

An examination of the literature on fertilizer experiments with the potato shows, however, that there are still many points on which further information is needed. In the past the results of potato fertilizer experiments have, for the most part, been based on yields alone. While this is probably the best way to measure the value of fertilizer mixtures, it affords but little opportunity to determine the influence of the component fertilizer elements on the development of the tuber itself. In order to obtain information on this point, the writers conducted a series of experiments to determine the effect of nitrogen, phosphoric acid, and potassium on (1) total weight of tubers per hill, (2) tuber length, width, and thickness, (3) number of tubers per plant, and (4) weight of individual tubers.

METHODS

The field experiments herein reported were conducted on the Penn loam type of soil [3] on the station farm at New Brunswick, N. J. The experimental area was divided into $\frac{1}{40}$-acre plots, 21 of which were fertilized according to the triangle method devised by Schreiner and Skinner (*11*).[4] In all cases the fertilizer mixtures carried 15 units of plant food; the three ingredients nitrogen (expressed as ammonia),

[1] Received for publication Feb. 2, 1931; issued August, 1931. Paper of the Journal Series, New Jersey Agricultural Experiment Station, Department of Plant Pathology.

[2] The writers wish to acknowledge their indebtedness to G. V. C. Houghland and F. R. Reid, of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, for assistance rendered in making the tuber measurements.

[3] The Penn soil type is typically a friable or mellow loam, having an Indian-red or brownish-red color. The surface soil ranges from 10 to 18 inches in depth and is underlain by an Indian-red clay loam. Both soil and subsoil occasionally contain from 5 to 20 per cent of sandstone fragments. The type is derived from fine-grained brown or red Triassic sandstone. The topography is gently rolling and the drainage is fair. The soil is considered almost equal in fertility to the associated limestone soils. The Penn loam type seems to be better adapted for general farming than for special crops. Corn, oats, wheat, grass, clover, and alfalfa do well on it. Applications of lime improve the land, particularly where alfalfa is to be grown. The soil responds readily to fertilizers, and is capable of producing satisfactory yields under proper methods of fertilization. It is easily cultivated and a good tilth can be maintained when reasonable care is taken to keep up a moderate supply of organic matter. The soil and subsoil are fairly retentive of moisture and crops do not suffer from dry weather. (Based on Soil Survey Report of York County, Pa., by J. O. Veatch, Lewis A. Hurst, and Gustavus B. Maynadier, 1914.)

[4] Reference is made by number (italic) to Literature Cited, p. 259.

phosphoric acid, and potash being used alone and in combinations of two and three of the constituents.[5] The nitrogen was derived equally from nitrate of soda and sulphate of ammonia. Superphosphate was used as the source of phosphoric acid (P_2O_5), and potassium sulphate as the source of potash (K_2O). In 1919–20 the experimental area was planted in alfalfa and in 1921 in corn. No fertilizer was applied to the alfalfa, but the corn received the same treatment as the potato crop which followed it, the application being made at the rate of 500 and 1,500 pounds per acre on the corn and potatoes, respectively. Since 1921 the various plots have been in potatoes continuously, and a winter cover crop of rye has been plowed under each spring.

The studies covered by this report were started in 1925, when 100 hills were dug by hand in each plot and all tubers, regardless of size, were harvested. In order to avoid differences which might arise in sampling, care was taken to plant seed pieces of uniform size, and all plots were cultivated alike. In each of the four years, certified Prince Edward Island Irish Cobbler seed potatoes were planted, and during the summer observations were made to determine to what extent virus diseases were present. Before the plants matured another careful examination was made, and 100 healthy plants, having two stems per hill and uniform as to size and general appearance, were staked in each plot for harvesting.

After the 100-hill samples were harvested, the remaining hills in the different plots were dug and total yields obtained. That the 100-hill sample is a fair criterion of the actual yield of the plots is apparent from a consideration of Figure 1. In this, the heavy line incloses the region of five fertilizer mixtures giving the greatest total yield in the case of the 100-hill sample, while the light line represents the same region in the case of total yields. The light and heavy broken lines, on the other hand, represent the five low-yielding fertilizer mixtures for total yields and the 100 hills respectively. It will be seen that these two areas differ but little in their distribution in the triangle. In view of this fact, 100 hills are believed to be a sufficiently large number upon which to base conclusions concerning the influence of the various fertilizer mixtures on yield

Immediately after the tubers were harvested, the number as well as the weight and measurements was secured for each of the 100 hills from the different plots. A count was obtained of all the tubers in each hill; those under 15 gm. were then weighed together while individual weights were obtained for each tuber weighing over 15 gm. All tubers weighing more than 40 gm. each were then measured to determine their length, width, and thickness.

The detailed results obtained from data on the 100-hill harvests were examined statistically to determine what reliance might be placed in the averages. The probable error of the experiment was calculated for the years covered by the test period, the method proposed by Student (*1*, *2*) being used. These constants are given in percentage in the various tables.

[5] See Figure 1 for triangular diagram showing fertilizer materials and their respective positions thereon. One of the earliest uses of the triangle method under field conditions was made by the office of Soil Fertility Investigations on potatoes in Aroostook County, Me. The field work was conducted under the supervision of B. E. Brown.

EFFECT OF SEASON ON TUBER DEVELOPMENT

In interpreting the results of fertilizer experiments with potatoes, it is important that consideration be given to rainfall and temperature, since these are very frequently limiting factors in production. In Table 1 and Figure 2 are shown the climatological data for New Brunswick for April to August, inclusive, for the four years in which this work was conducted.

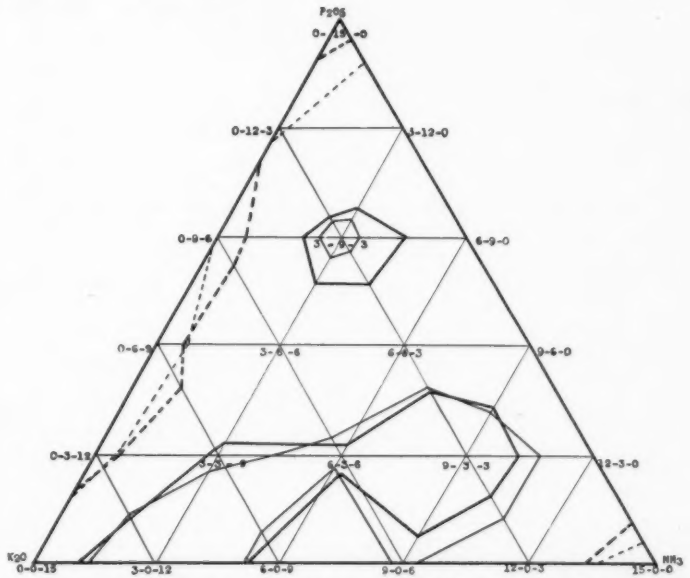


FIGURE 1.—Relation between total plot yields and yields of the 100-hill units. For potatoes when grown with different fertilizer mixtures. The light solid and light broken lines indicate the regions of high and low yields in the case of total plot yields, while the heavy solid and heavy broken lines indicate the regions of high and low yields in the case of the 100-hill samples

The 4-year period includes two seasons with relatively low rainfall (1925 and 1926), one with average rainfall (1927), and one with abundant rainfall (1928). Temperature conditions were more uniform than rainfall in each of the four years, the mean daily temperatures rising rather uniformly from approximately 45° F. the 1st of April to about 80° by the end of July. The most noteworthy variation was the abnormally high temperatures during the first half of June in 1925. These were accompanied by high evaporation rates and heat units and a deficiency in rainfall.

TABLE 1.—*Climatological data, New Brunswick, N. J., 1925–1928*

TOTAL MONTHLY PRECIPITATION IN INCHES

Year	April	May	June	July	August
1925	2.80	2.94	2.01	4.36	2.07
1926	1.79	2.11	2.70	4.62	8.30
1927	2.84	3.72	2.80	6.98	9.62
1928	4.32	1.91	7.18	8.66	5.87
Average	2.94	2.67	3.67	6.15	6.46

MONTHLY MEAN TEMPERATURE IN DEGREES FAHRENHEIT

1925	51.6	57.8	74.7	72.6	71.2
1926	46.7	59.8	64.9	73.7	72.8
1927	47.9	58.3	66.1	73.2	67.4
1928	49.0	59.2	68.0	75.7	75.3
Average	48.8	58.8	68.4	73.8	71.7

TOTAL MONTHLY HEAT UNITS (TEMPERATURE OVER 45° F.) [a]

1925	198	397	891	856	812
1926	51	459	597	300	862
1927	87	412	633	874	694
1928	120	440	690	952	939
Average	114.00	427.00	702.75	893.00	826.75

EVAPORATION FROM FREE WATER SURFACE IN INCHES [b]

1925	3.915	4.559	7.079	5.234	4.884
1926	3.973	6.116	4.890	5.430	4.030
1927	4.045	4.690	5.930	5.780	4.310
1928	[c] 3.842	5.030	4.575	5.020	4.860
Average	3.944	5.099	5.618	5.366	4.521

[a] 1 heat unit equals 1° F. above a mean daily temperature of 45° F. Thus a mean daily temperature of 48° F. may be expressed as 3 heat units, or 3 units of effective temperature for plant growth. The sum of the daily heat units gives the total for the month.
[b] At Runyon, N. J.
[c] Estimated.

TABLE 2.—*Average yields and weights per hill of potatoes grown in 1925–1928*

Year	All tubers			Tubers over 40 grams in weight				Tubers 75 grams in weight or over			
	Tubers per hill	Weight per hill	Weight per tuber	Tubers per hill	Weight per hill	Percentage by weight of all tubers	Weight per tuber	Tubers per hill	Weight per hill	Percentage by weight of all tubers	Weight per tuber
	Number	*Grams*	*Grams*	*Number*	*Grams*		*Grams*	*Number*	*Grams*		*Grams*
1925	3.77	154.22	41.9	1.60	117.0	75.86	72.42				
1926	5.40	321.83	59.8	3.55	287.0	89.18	80.85	1.75	182.72	56.77	103.9
1927	4.83	281.79	58.0	3.03	250.5	88.90	81.53	1.54	162.83	57.79	103.9
1928	6.34	381.19	59.8	3.83	336.3	88.22	86.67	2.15	240.43	63.07	110.0
Average	5.08	284.76	54.9	3.00	247.7	85.54	80.37	1.81	195.33	59.21	105.9

The average yields for all plots for the 4-year period are shown in Table 2. The planting and harvesting dates for the four years were as follows: In 1925, April 9 and August 13; 1926, April 23 and August

21; 1927, April 18 and July 25; 1928, April 17 and August 13. The yields were highest in 1928 followed by those of 1926, 1927, and 1925. In comparing the rainfall and average yields per hill in 1925 and 1926, it will be seen from Tables 1 and 2 that while the total precipitation for April, May, June, and July was greater in the former than in the latter year, the average yield per hill in 1925 was less than half that in 1926. It is doubtful whether the rainfall during August was of much importance in determining yields, owing to the stage of maturity of the vines and tubers.

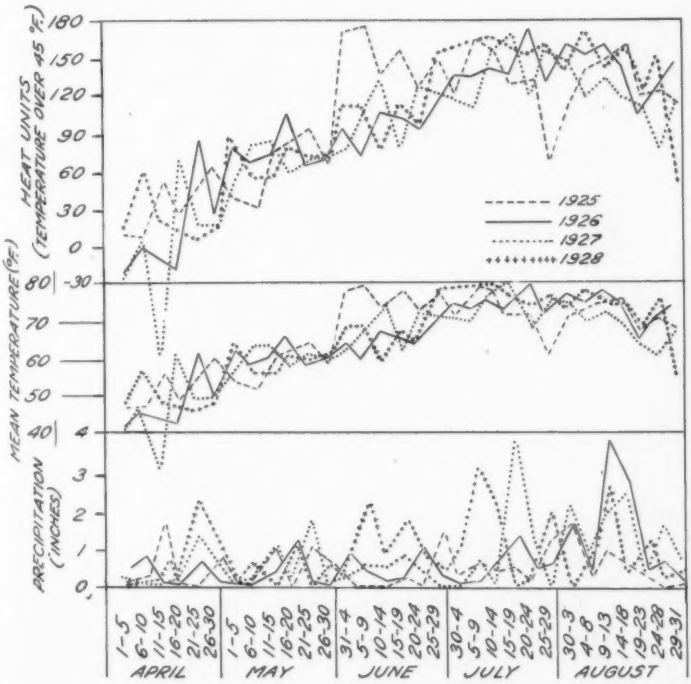


FIGURE 2.—Precipitation, mean temperature, and heat unit data for New Brunswick, N. J., during the months from April to August, inclusive, 1925–1928

The average yield per hill in 1927 was lower than in 1926. The crop matured early in 1927; this can not, however, be explained on the ground of insufficient moisture since the total amount during April, May, and June was greater than in 1926. The mean temperature in June of 1927 was slightly higher than in June of 1926, particularly during the early part of the month. The total heat units for this period were likewise higher and the evaporation rates were considerably higher. These conditions, no doubt, account in part, at least, for the lower yields obtained in 1927.

The largest yields were obtained in 1928. Rainfall was abundant throughout the growing season, and while the mean temperature for

June and July was slightly higher than in either 1926 or 1927 this was probably offset by an ample supply of moisture and a lower rate of evaporation.

Total yields are determined both by the number and weight of tubers. In 1925 both the number of tubers per hill and the average weight per tuber were well below the 4-year average, whereas in the other three years the average weight per tuber was nearly constant, while the number of tubers per hill varied directly with total yields. The unusually high temperatures during early June in 1925, together with a deficiency in moisture, no doubt account for the low average weight per tuber as well as for the small number of tubers. It was shown by Clark (*4*) that both yield and number of tubers were increased by irrigation, particularly where irrigation was started before, rather than after, the tubers had begun to set. Hardenburg (*5*) found that soil moisture exercised a less marked influence than air temperature on tuber set, but an increase in both the weight of tubers per hill and the average tuber weight followed increases in soil moisture. It is difficult to explain the difference in number of tubers produced in 1925, 1926, and 1927 on the basis of either moisture or temperature. The average number of tubers per plant in 1927 was slightly less than in 1926, despite the fact that rainfall was greater in the former years. It should be recalled, however, that the mean temperature in June in 1927 was slightly higher than in 1926, and the evaporation rates in this year were higher than in either 1926 or 1928. The largest number of tubers per hill as well as the largest tubers were produced in 1928. The precipitation in this year, particularly during June and July, was unusually high, and while the mean temperature was likewise high, the evaporation rates were lower than in any other of the four years considered.

The number of tubers which attained a weight of 40 gm. or more in 1925 was 46.6 per cent less than the 4-year average, while the difference in percentage by weight of this class was only 9.68 less than the 4-year average. In comparing the results for the other three years it will be seen that the percentage by weight of all tubers varies but little, whereas there is an appreciable difference when the percentage by number is considered. It is clear from a consideration of the four years that while tuber size was modified somewhat by weather conditions, the factor that contributed most to low yields was the difference in the number of tubers per hill. It is evident that a deficiency of rainfall during the critical period of tuber development, together with high mean temperatures, resulted in the production of few tubers. On the other hand, fairly high temperatures together with an abundance of moisture, as in 1928, resulted in the production of a comparatively large number of tubers.

The number of tubers which attained a weight of 75 gm. or more in 1925 was small, and the data for that year were therefore excluded from consideration. In the other three years it will be seen that the same relationship exists as in the case of the tubers weighing over 40 gm.; that is, the differences in total yield resulted mainly from differences in number of tubers rather than from variations in their average weight.

EFFECT OF SEASON AND OF DIFFERENT FERTILIZER MIXTURES ON TUBER SHAPE

The work described in this section was done in 1926, 1927, and 1928. Only those tubers weighing 75 gm. or more are included in this study. As previously indicated, measurements were made of tuber width, thickness, and length. Before discussing the influence of fertilizers on tuber shape, it is necessary to consider the influence of seasonal conditions, since it has been reported that soil moisture and temperature may influence shape.

The average measurements of all tubers weighing over 75 gm. for each of the three years are presented in Table 3.

TABLE 3.—*Dimensions and shape indexes of potatoes grown in 1926–1928*

Year	Average length	Average width	Average thickness	Average shape index *
	cm.	*cm.*	*cm.*	
1926	5.84	5.80	4.94	1.09
1927	5.79	5.69	4.95	1.09
1928	6.31	5.72	4.88	1.19
Average	5.98	5.74	4.92	1.12

* Shape index $= \frac{2 \times \text{length}}{\text{width} + \text{thickness}}$.

The longest tubers were produced in 1928, the next longest in 1926, and the shortest in 1927; the widest were produced in 1926, the next widest in 1928, and the narrowest in 1927. The average tuber thickness was approximately the same in 1926 and 1927 and was slightly greater than in 1928. Tuber index values were greatest in 1928, the index values in 1926 and 1927 being identical. In view of the fact that the rainfall in 1928 was greater than in the other two years, it would seem that moisture is an important factor in influencing tuber length. The average shape indexes for the different plots for each of the three years is shown in Table 4. The shape index was calculated by the formula: $\frac{2 \times \text{length}}{\text{width} + \text{thickness}}$. In this table the five highest and five lowest indexes are indicated by bold-type and italics, respectively. Of the five high average shape index values three were high in each of the three years, while the remaining two were high in two of the three years. The only outstanding variations are in the case of the 0–15–0 and 0–12–3 mixtures. The former was included among the five low in 1926 and the five high in 1927, while the latter was included among the five high in 1926 and the five low in 1928. Of the five lowest shape indexes, four were the averages of the same fertilizer mixtures for two and one for three years.

TABLE 4.—*Potato tuber shape index values for the years 1926–1928*

Treatment No.	Fertilizer mixtures used	Tuber shape index values [a]			
		1926	1927	1928	Average
	No fertilizer	1.11	1.11	1.15	1.12
1	0–15–0	*1.06*	**1.18**	1.20	1.15
2	0–12–3	**1.11**	1.11	*1.16*	1.13
3	3–12–0	**1.16**	1.10	**1.27**	**1.18**
4	0–9–6	1.07	*1.03*	*1.11*	*1.07*
5	3–9–3	1.09	1.09	1.21	1.13
6	6–9–0	**1.13**	**1.13**	**1.27**	**1.18**
7	0–6–9	1.08	*1.01*	*1.12*	*1.07*
8	3–6–6	1.07	1.07	1.17	1.10
9	6–6–3	1.09	1.07	1.22	1.13
10	9–6–0	**1.12**	**1.12**	**1.23**	**1.16**
11	0–3–12	*1.06*	*1.04*	*1.12*	*1.07*
12	3–3–9	*1.06*	*1.00*	1.17	*1.08*
13	6–3–6	1.10	1.10	1.21	1.14
14	9–3–3	1.10	1.07	1.23	1.13
15	12–3–0	**1.15**	**1.12**	**1.25**	**1.17**
16	0–0–15	*1.02*	1.08	*1.14*	*1.08*
17	3–0–12	*1.01*	1.12	1.18	1.10
18	6–0–9	1.08	1.07	1.19	1.11
19	9–0–6	*1.04*	1.08	1.22	1.11
20	12–0–3	*1.04*	*1.07*	1.19	1.10
21	15–0–0	1.07	**1.15**	**1.25**	**1.16**

[a] Probable error of experiment, 1.04 per cent.

Table 4 shows that a good correlation exists between the potash content of the fertilizer and tuber index values. Of the 21 fertilizer mixtures reported, 6 contain no potash. These without exception have the highest average index values. There are 10 fertilizer mixtures in which the potash content is 6 per cent or more; and these, with 3 exceptions, include all of the lowest index values. This seems to indicate that potash is the element most directly related to index values. The absence of nitrogen is also directly associated with low index values.

As previously pointed out, the average weight of tubers in the 75-gm. class was slightly greater in 1928 than in the other two years. This no doubt accounts for the fact that the tuber index values were the highest in this year, since, as Neumann (*8*) has shown, length is the dominating dimension when shape ratios are compared with tuber size, indicating that increase in tuber size is largely caused by increase in length.

The results of the tuber measurements are shown in Table 5. The average width measurements are plotted on the triangle in Figure 3. In this figure the six fertilizer mixtures that produced the widest and the five that produced the narrowest tubers are inclosed in the areas designated by crosses and circles, respectively. It will be observed that the mixtures that produced the narrow tubers are characterized by high phosphoric acid, with a range from 0 to 6 per cent nitrogen and from 0 to 6 per cent potash, the narrowest tubers being produced by the 0–15–0 mixture. Of the 6 mixtures that produced the widest tubers, 4 carried none and 2 carried 3 per cent phosphoric acid, while the nitrogen content varied from 0 to 12 per cent and the potash from 0 to 15 per cent.

TABLE 5.—*Dimensions in centimeters of potato tubers weighing over 75 gm. grown 1926 to 1928*

Fertilizer mixture	Average width				Average thickness				Average length			
	1926	1927	1928	Average	1926	1927	1928	Average	1926	1927	1928	Average
Checks	5.65	5.28	5.64	5.52	4.92	4.59	4.85	4.79	5.90	5.47	6.03	5.80
0-15-0	5.67	5.10	5.69	5.49	4.86	4.38	4.84	4.69	5.60	5.62	6.31	5.84
0-12-3	5.66	5.60	5.64	5.63	4.90	4.91	4.80	4.87	5.85	5.85	6.04	5.91
3-12-0	5.63	5.65	5.67	5.65	4.89	4.88	4.76	4.84	6.13	5.78	6.63	6.18
0-9-6	5.70	5.67	5.74	5.70	4.86	4.96	4.80	4.87	5.68	5.48	5.86	5.67
3-9-3	5.75	5.69	5.80	5.75	4.91	4.98	4.99	4.96	5.80	5.80	6.55	6.05
6-9-0	5.80	5.50	5.66	5.65	4.95	4.87	4.85	4.89	6.08	5.87	6.70	6.22
0-6-9	5.82	5.70	5.63	5.72	4.96	4.97	4.78	4.90	5.84	5.37	5.82	5.68
3-6-6	5.79	5.78	5.71	5.76	4.96	5.05	4.87	4.96	5.73	5.79	6.21	5.91
6-6-3	5.80	5.66	5.76	5.74	4.97	5.02	4.94	4.98	5.86	5.74	6.54	6.05
9-6-0	5.82	5.67	5.74	5.74	4.97	4.99	4.95	4.97	6.04	5.95	6.58	6.19
0-3-12	5.77	5.93	5.66	5.79	4.93	5.13	4.78	4.95	5.67	5.77	5.84	5.76
3-3-9	5.81	5.83	5.76	5.80	5.03	5.09	4.96	5.03	5.73	5.48	6.30	5.84
6-3-6	5.86	5.79	5.76	5.80	5.06	5.11	4.99	5.05	6.00	6.01	6.50	6.17
9-3-3	5.97	5.89	5.82	5.89	5.00	5.17	4.92	5.03	6.06	5.93	6.62	6.20
12-3-0	5.95	5.86	5.84	5.88	4.87	5.10	4.93	4.97	6.25	6.15	6.71	6.37
0-0-15	5.96	5.84	5.76	5.85	5.08	5.08	4.83	5.00	5.64	5.91	6.02	5.86
3-0-12	5.91	6.12	5.70	5.91	5.06	5.18	4.86	5.03	5.53	6.31	6.24	6.03
6-0-9	5.81	5.90	5.81	5.84	4.92	5.11	4.96	5.00	5.82	5.89	6.39	6.03
9-0-6	5.95	6.02	5.77	5.91	4.97	5.12	4.90	5.00	5.69	6.01	6.50	6.07
12-0-3	5.95	5.91	5.74	5.87	4.83	5.07	4.89	4.93	5.62	5.86	6.35	5.94
15-0-0	5.88	5.64	5.78	5.77	4.95	4.91	4.89	4.92	5.82	6.07	6.66	6.18

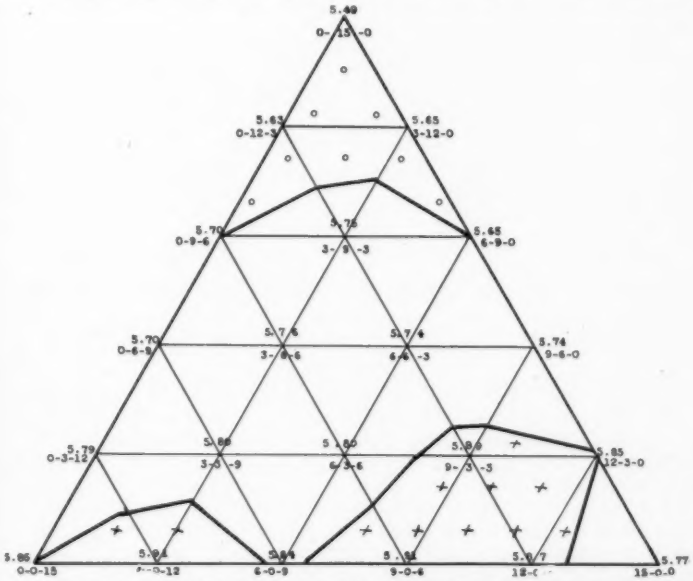


FIGURE 3.—Average widths of potato tubers weighing 75 gm. or more when grown with different fertilizer mixtures. Areas of the widest tubers are indicated by crosses, those of the narrowest by circles

A similar relationship obtains for tuber thickness. These data, presented in Figure 4, show that the five fertilizer mixtures that produced the thinner potatoes are identical with those that produced

the narrowest tubers. While there are only slight differences in the region producing widest and that producing thickest tubers, there is a tendency for the region producing the latter to move away from the high-nitrogen side of the triangle in the direction of higher potash and phosphoric acid. In the case of both the wider and thicker potatoes it will be observed also that, with but one exception, all the mixtures included in the high areas carried both nitrogen and potash while all the mixtures included in the low areas were deficient in one or the other of these elements.

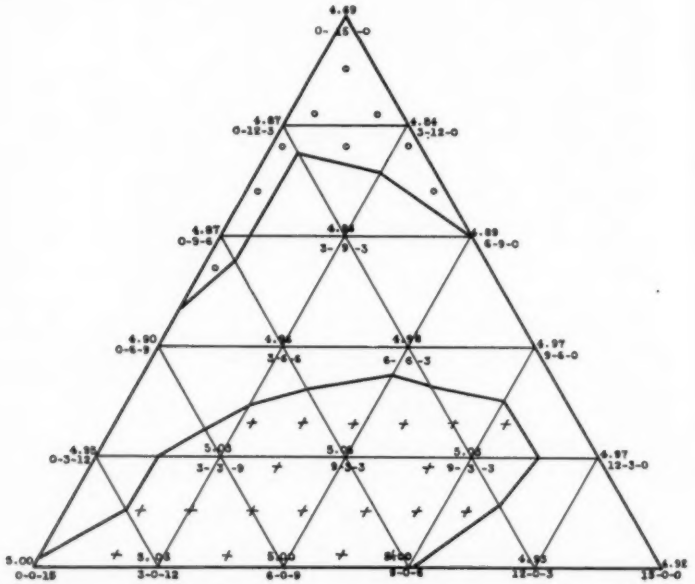


FIGURE 4.—Average thicknesses of potato tubers weighing 75 gm. or more when grown with different fertilizer mixtures. Areas of the widest tubers are indicated by crosses, those of the narrowest by circles

The average length of the tubers is shown in Figure 5. Five of the six fertilizer mixtures that produced the longest tubers carried no potash, and the other carried only 3 per cent. The nitrogen content of these mixtures ranged from 3 to 15 per cent and the phosphoric acid from 0 to 12 per cent. The longest tubers were produced by the 12–3–0 mixture. Of the five fertilizers that produced the shortest tubers, one carried 3 per cent nitrogen and the others none. The phosphoric acid content of these five mixtures ranged from 3 to 15 and the potash from 0 to 12 per cent.

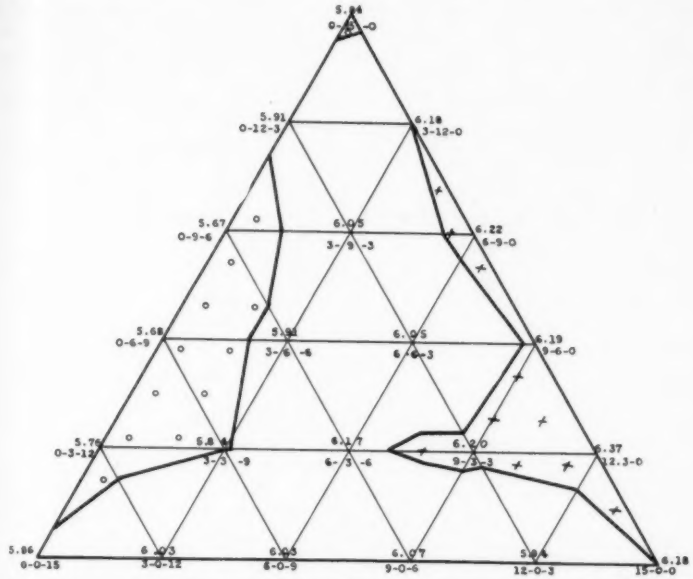


FIGURE 5.—Average lengths of potato tubers weighing 75 gm. or more when grown with different fertilizer mixtures. Areas of the longest tubers are indicated by crosses, those of the shortest by circles

TABLE 6.—*Effect of range and average of fertilizer ratios upon high and low values for width, thickness, length, and tuber index of potatoes*

Item	Average measurement	Fertilizer mixtures, giving highest tuber measurements					
		Range			Average		
		NH_3	P_2O_5	K_2O	NH_3	P_2O_5	K_2O
Width.....centimeters	5.88	0–12	0–3	0–15	9	1.2	4.8
Thickness.....do	5.03	0–9	0–3	3–15	5.4	1.8	7.8
Length.....do	6.23	3–15	0–12	0–3	7.8	6.6	.6
Index value.....do	1.17	3–15	0–12	0	9	6	0

Item	Average measurement	Fertilizer mixtures, giving lowest tuber measurements					
		Range			Average		
		NH_3	P_2O_5	K_2O	NH_3	P_2O_5	K_2O
Width.....centimeters	5.62	0.6	9–15	0–6	1.8	11.4	1.8
Thickness.....do	4.83	0–6	9–15	0–6	1.8	11.4	1.8
Length.....do	5.76	0–3	3–15	0–12	0.6	4.2	10.2
Index value.....do	1.07	0–3	0–9	6–15	0.6	4.2	10.2

In order to show better the influence of nitrogen, phosphoric acid, and potash on tuber shape, the five mixtures having high and low

values for width, thickness, length, and tuber index values are given in Table 6. The table shows the range of the three ingredients as well as their average values. In the case of widest tubers, while there is a wide range in both the nitrogen and potash content of the five high mixtures, the tendency is toward higher nitrogen, the average nitrogen content being 9 per cent as compared with 4.8 per cent for potash. Phosphoric acid was present in these mixtures only in small amounts. The average mixture producing the thicker tubers is lower in nitrogen and higher in potash than the average mixture producing the wider tubers. There is, however, but little difference in the phosphoric acid content of the mixtures. The narrower tubers were produced by mixtures carrying large amounts of phosphoric

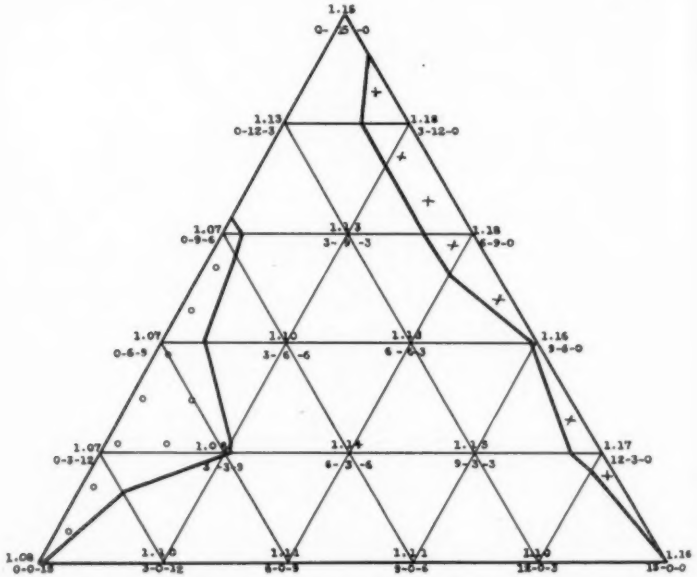


FIGURE 6.—Tuber index values of potatoes when grown with different fertilizer mixtures. Areas of the highest values are indicated by crosses, those of the lowest by circles

acid with small amounts of nitrogen and potash, and these are never present together. The thinner tubers were produced by the same fertilizer mixtures as the narrower ones, i. e., low nitrogen and potash with high phosphoric acid.

The length of the tubers and their shape index may be considered together, since these occupy the same general position on the triangle in both their high and low values. The tuber index values are shown in Figure 6. Tuber index is probably the most important criterion by which to judge the influence of the three nutrients on tuber shape. It is apparent from the location of the high and low areas on the figure than an abundance of nitrogen, together with a deficiency of potash, results in the production of tubers which are longer and which, consequently, have a higher index value than those produced by

fertilizers having an abundance of potash and small amounts of nitrogen.

Several workers have reported that environmental conditions influence tuber shape. Werner (*12*) found that in the case of both healthy and spindle-tuber stock, the tubers of the Triumph variety tended to grow longer in heavy or fine-textured soils than in more sandy soils. Longer tubers were likewise found to be produced in soils of high rather than of low moisture content. Hardenberg (*6*) reports that in the case of both the Green Mountain and Rural varieties the tubers were more elongated and flatter in the lighter soils. He concludes that while varieties differ in their response to type of soil, the soil factor exhibits a distinct influence on the shape of the tuber. Krantz (*7*) grew the Early Ohio variety in a sandy loam and in a clay loam. In the former the tubers were elongated and cylindrical; in the latter they were relatively thicker, broader, and shorter. Neumann (*8*) reports that potatoes grown in sandstone soil were short, while those grown in heavy soils were long. He attributes these differences in shape to differences in aeration, a soil with abundant aeration producing short tubers.

It is possible that the differences in shape which result from changes in environmental conditions may be due in part to modified nutrient conditions. This has been found to be true for certain other crops. In the case of the sweetpotato, for example, it was generally believed that marked differences in shape were due to such factors as soil type, depth of plowing, etc. The work of Schermerhorn (*10*) and of Robbins et al. (*9*) has demonstrated, however, that these changes in shape result from a modification of the soil nutrients. When potassium was lacking and sufficient nitrogen was present, the potatoes grew long, but the reverse was true when an adequate supply of potassium was present in the nutrient solution.

Neumann (*8*) conducted greenhouse and field tests to determine the influence of nitrogen, phosphoric acid, and potash on tuber shape. He concludes that nitrogen has a lengthening effect on the tubers while development in thickness is favored by potash and phosphorus. In his experiments, a comparison was made of three fertilizer combinations, K+P, K+P+N, and 3 (K+P) 2N. In one test where potatoes were grown in cultures maintained at 40 per cent moisture and fertilized with K+P, the tuber length was 5.70 ± 0.36 as compared with 7.00 ± 0.57 for the cultures fertilized with K+P+N. On the other hand, where *3* (K+P) 2N was used, the tuber length was reduced as compared with that when K+P+N or K+P was used. In still another series of pot cultures, tubers produced in the series receiving nitrogen were longer than those in the no-nitrogen series. In field tests where a similar comparison was made on four different soil types, longer tubers were produced in three cases as a result of using nitrogen; while in the fourth case, a red clay, no difference in length was noted. Neumann does not attempt to explain this, but states that in general, the use of nitrogen on the red clay had no influence on tuber shape.

In the light of the results obtained in extensive investigations to determine the influence of nitrogen and phosphorus on the development of the plant cell, Neumann's conclusion that nitrogen is the cause of tuber elongation would hardly appear to be tenable. Rob-

bins et al. (loc. cit.) state that an adequate supply of potassium appears to be essential for the production of "chunky" sweetpotatoes. In the absence of a sufficient amount of this element cell division in the direction of the diameter of the potato is retarded and long sweetpotatoes result, even though there may be an abundance of nitrate nitrogen and carbohydrates in the plant tissue and nitrate in the nutrient solution. Similar relationships have been shown with other plants, and the available evidence indicates that where only a small amount of potassium is present in the plant it moves to the more actively growing parts, and consequently cell division proceeds normally at the growing tip only, with the result that growth in length results at the expense of development in diameter. On the other hand, when the supply of potassium in the plant is adequate, cell division proceeds normally in all directions and development in diameter keeps pace with length, and a thicker plant part results.

The data here presented indicate that this same relationship holds true for the potato tuber. In comparing shape indexes, for example, it is apparent that, without exception, the tubers having the highest index, i. e., the longest tubers, were produced by fertilizer mixtures high in nitrogen with little or no potash. (Fig. 6.) On the other hand, the mixtures producing the shorter tubers were characterized by comparatively high potassium with little or no nitrogen. As previously indicated, a green cover crop of rye was plowed under each year on the plots used in this experiment, so that small amounts of nitrogen at least were available, whereas there is little doubt but that the supply of available potash was largely exhausted.

From these results, it appears reasonable to assume that potassium is the plant-food element that is chiefly influential in determining tuber shape. Where the soil is deficient in this element, and an adequate supply of nitrogen is present, cell division will proceed at the tip of the tuber at a faster rate than in the direction of the diameter and a longer tuber will result, but when sufficient potassium is present, relatively shorter tubers will be produced because of the more general development of the cells in the short axis of the tuber.

EFFECT OF DIFFERENT FERTILIZER MIXTURES ON YIELDS

ALL TUBERS

The influence of the different fertilizer mixtures on yield, number, and weight of all tubers is shown in Table 7. The average yield of the check plots was 228.9 gm., while the average for the 21 fertilizer mixtures ranged from 242.8 to 354.7 gm. per hill. The average total yields from the different treatments are plotted on the triangle in Figure 7. The five fertilizer mixtures producing highest yields were 3–0–12, 9–3–3, 3–3–9, 6–3–6, and 3–9–3, while those producing low est yields were 15–0–0, 0–3–12, 0–9–6, 0–6–9, and 0–15–0. It is apparent that fertilizers deficient in nitrogen or potash or both are associated with low yields, while fertilizers containing both nitrogen and potash produce relatively high yields. It is likewise apparent that in this particular soil type phosphoric acid is of relatively minor importance in determining yields.

TABLE 7.—*Influence of different fertilizer mixtures on average, number and weight per hill, and average weight of potato tubers* [a]

Fertilizer mixture treatment used	Average number of tubers per hill [b]	Average weight per hill [c]	Average weight per tuber [d]	Fertilizer mixture treatment used	Average number of tubers per hill [b]	Average weight per hill [c]	Average weight per tuber [d]
		Grams	*Grams*			*Grams*	*Grams*
Checks	4.88	228.93	46.1	0-3-12	5.12	244.79	47.6
0-15-0	5.16	264.38	50.1	3-3-9	5.73	319.96	55.1
0-12-3	4.94	270.68	53.5	6-3-6	5.36	318.35	57.6
3-12-0	5.23	292.01	54.1	9-3-3	5.04	329.18	63.5
0-9-6	5.01	251.08	49.4	12-3-0	4.53	302.51	64.7
3-9-3	5.47	317.98	56.5	0-0-15	5.11	293.27	56.6
6-9-0	4.84	278.52	55.3	3-0-12	5.69	354.73	61.9
0-6-9	5.49	253.23	46.1	6-0-9	5.24	304.86	56.2
3-6-6	5.74	304.87	52.9	9-0-6	4.99	315.92	62.2
6-6-3	4.96	310.36	59.6	12-0-3	4.67	281.24	60.8
9-6-0	4.90	296.68	57.6	15-0-0	4.14	242.83	57.3

[a] Derived from 4 yearly averages.
[b] Probable error, 3.48 per cent.
[c] Probable error, 4.70 per cent.
[d] Probable error, 4.01 per cent.

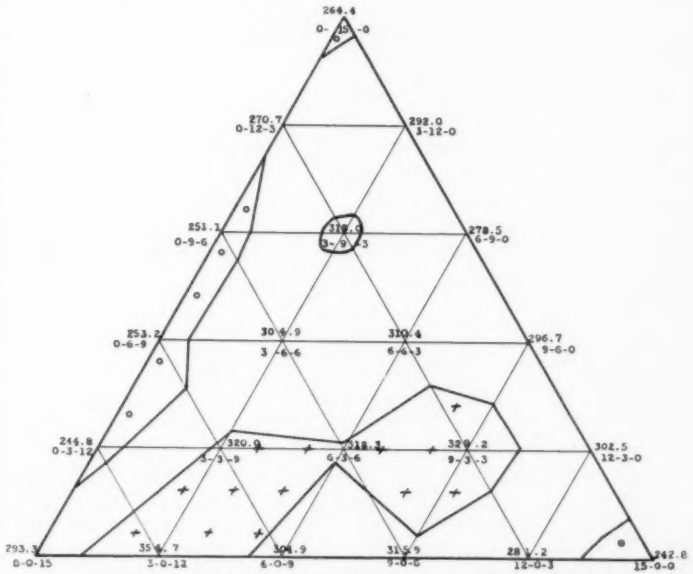


FIGURE 7.—Total weight per hill of potatoes when grown with different fertilizer mixtures. Areas of the largest yields are indicated by crosses, those of the smallest by circles

The total number of all tubers per hill for the different treatments is shown in Figure 8. The average of the check was 4.88. The largest number of tubers, 5.74, was produced by the 3–6–6 mixture; the remaining four mixtures included in the high group were 3–3–9, 3–0–12, 0–6–9, and 3–9–3. Fewest tubers were produced by the 15–0–0 mixture, followed by the 12–3–0, 12–0–3, 6–9–0, and 9–6–0 mixtures. It is apparent that the greatest number of tubers was in most cases produced by fertilizer high in potash and low in nitrogen; while smaller numbers were associated with fertilizers high in nitro-

gen with little or no potash. The phosphoric acid content of the five high and five low fertilizer mixtures varied but little.

The tubers from the check plots averaged 46.1 gm. each, while the tubers from the fertilized plots ranged in weight from 46.1 to 64.7 gm. The weight per tuber for the different plots is presented in Figure 9. The five mixtures producing the largest tubers were 12–3–0, 9–3–3, 9–0–6, 3–0–12, and 12–0–3; those producing the smallest tubers were 0–6–9, 0–3–12, 0–9–6, 0–15–0, and 3–6–6. It will be observed that, with but one exception (the 3–0–12 mixture), the largest tubers were produced by fertilizers carrying large amounts of nitrogen with none to small amounts of phosphoric acid and potash. It should be noted that the largest total yield of tubers was likewise produced by the 3–0–12 mixture.

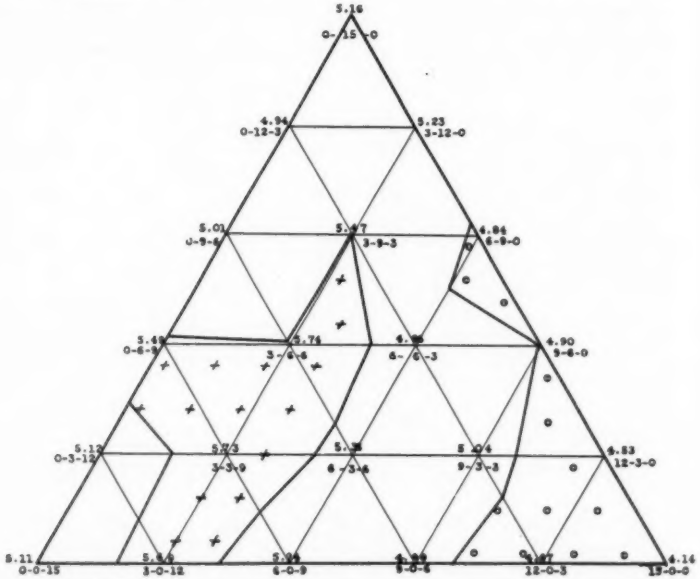


FIGURE 8.—Total number of tubers per hill of potatoes when grown with different fertilizer mixtures. Areas of the largest number of tubers are indicated by crosses, those of the smallest by circles

In order to show better the influence of the fertilizer mixtures on total yields as well as on number and size of all tubers, the five high and five low fertilizer mixtures in the case of each of these are summarized in Table 8. Since total yields are determined both by the number of tubers and by their average weight, it is not surprising to find that the fertilizer mixtures producing largest total yields have certain characteristics in common with those producing the largest number of tubers as well as the largest tubers. Thus, although the greatest number of tubers was produced by fertilizers high in potash and the largest tubers by those high in nitrogen, both nitrogen and potash were present in relatively large amounts in those mixtures producing the largest yields. The five mixtures producing highest

total yields contained nitrogen and potash in every mixture, while the five mixtures producing low yields are characterized by the absence of one, or both, of these elements.

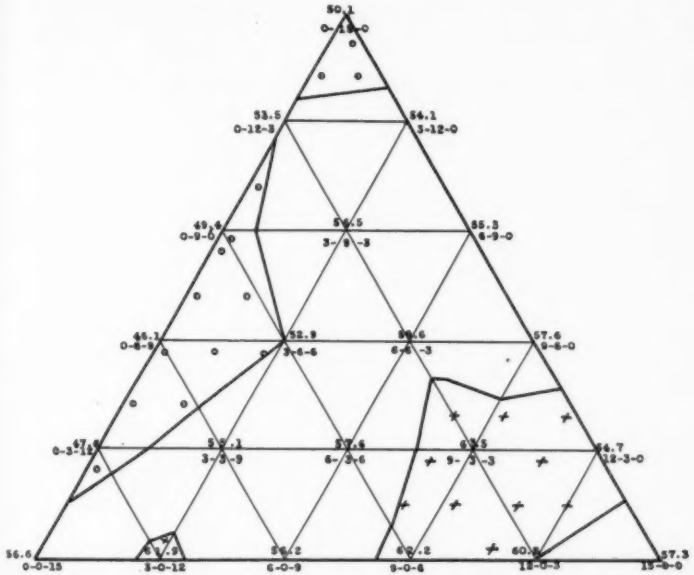


FIGURE 9.—Average weight per tuber of potatoes when grown with different fertilizer mixtures. Areas of the largest tubers are indicated by crosses, those of the smallest by circles

TABLE 8.—*Effect of fertilizer mixtures upon the weight per hill, number of tubers, and weight per tuber of potatoes*

High-yielding fertilizer mixtures	Yield per hill	Low-yielding fertilizer mixtures	Yield per hill	High-yielding fertilizer mixtures	Tubers
	Grams		*Grams*		*Number*
3-0-12	354.7	15-0-0	242.8	3-6-6	5.74
9-3-3	329.2	0-3-12	244.8	3-3-9	5.73
3-3-9	320.0	0-9-6	251.1	3-0-12	5.69
6-3-6	318.3	0-6-9	253.2	0-6-9	5.49
3-9-3	318.0	0-15-0	264.4	3-9-3	5.47

Low-yielding fertilizer mixtures	Tubers	High-yielding fertilizer mixtures	Weight per tuber	Low-yielding fertilizer mixtures	Weight per tuber
	Number		*Grams*		*Grams*
15-0-0	4.14	12-3-0	64.7	0-6-9	46.1
12-3-0	4.53	9-3-3	63.5	0-3-12	47.6
12-0-3	4.67	9-0-6	62.2	0-9-6	49.4
6-9-0	4.84	3-0-12	61.9	0-15-0	50.1
9-6-0	4.90	12-0-3	60.8	3-6-6	52.9

From a consideration of the fertilizers producing largest tubers it is clear that phosphoric acid is of minor importance in determining tuber weight, since it is present in only three of the five high mixtures. That it is more important in determining tuber number is apparent

from the fact that four of the five mixtures producing the largest number of tubers carried phosphoric acid. The relative importance of phosphoric acid may be seen from a consideration of the average of the five high mixtures in each of the three groups under consideration. In the case of the tuber weights the average phosphoric acid content of the five mixtures is 1.2 per cent, while the average in the case of total number of tubers is 4.8 per cent. It would appear that this element is of greater importance in determining the number of tubers produced than their average weight.

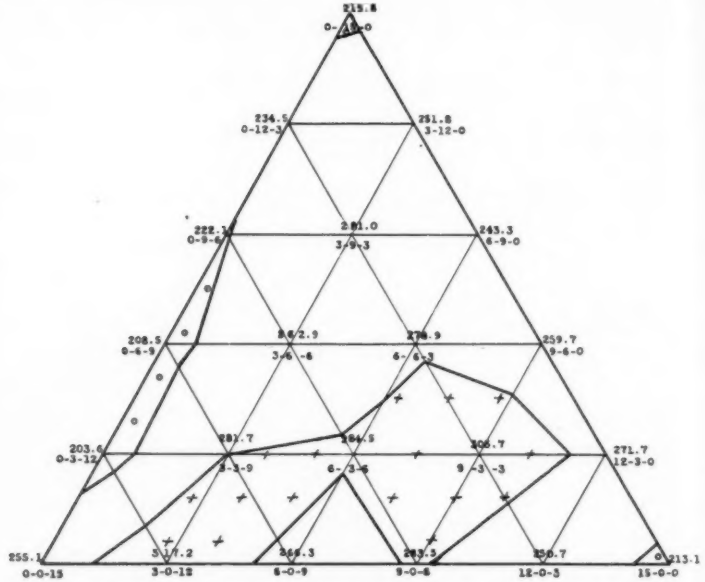


FIGURE 10.—Total yield of potato tubers weighing 40 gm. or more when grown with different fertilizer mixtures. Areas of the highest yields are indicated by crosses, those of the lowest by circles

TUBERS WEIGHING MORE THAN 40 GM

The influence of nitrogen, phosphoric acid, and potash on yield per hill of tubers weighing over 40 gm. as well as on their number and average weight, is shown in Table 9. The average yield of the check plots was 183.0 gm. and that of the high and low yielding fertilizers 317.2 and 203.6 gm. respectively.

The total yields of the tubers in the 40-gm. class are shown in the triangle in Figure 10. The region on the triangle including the five mixtures producing largest yields is characterized by medium to high nitrogen and potash with none too low phosphoric acid, the five most productive mixtures being, 3–0–12, 9–3–3, 6–3–6, 9–0–6, and 3–3–9. The five low-yielding mixtures are without nitrogen or potash, or both, and the phosphoric acid content varies from 0 to 15 per cent.

TABLE 9.—*Influence of different fertilizer mixtures on average, number, and weight per hill, and average weight of potato tubers weighing 40 gm. or more*[a]

Fertilizer mixture used	Average number tubers per hill [b]	Average weight per hill [c]	Average weight per tuber [d]	Fertilizer mixture used	Average number tubers per hill [b]	Average weight per hill [c]	Average weight per tuber [d]
		Grams	*Grams*			*Grams*	*Grams*
Check	2.45	183.0	71.96	0-3-12	2.70	203.6	75.39
0-15-0	2.84	219.8	75.05	3-3-9	3.40	281.7	81.24
0-12-3	2.93	234.5	78.57	6-3-6	3.20	284.5	86.79
3-12-0	3.13	251.8	78.21	9-3-3	3.36	305.7	89.45
0-9-6	2.81	222.1	77.81	12-3-0	2.96	271.7	89.65
3-9-3	3.45	281.0	79.41	0-0-15	3.04	255.1	82.08
6-9-0	2.97	243.3	78.85	3-0-12	3.62	317.2	86.71
0-6-9	2.71	208.5	77.17	6-0-9	3.21	266.3	80.86
3-6-6	3.31	262.9	78.41	9-0-6	3.25	283.5	85.61
6-6-3	3.25	278.9	83.32	12-0-3	2.96	250.7	84.59
9-6-0	3.05	259.7	82.28	15-0-0	2.57	213.1	81.44

[a] Derived from 4 yearly averages.
[b] Probable error, 4.37 per cent.
[c] Probable error, 6.19 per cent.
[d] Probable error, 3.41 per cent.

The number of tubers weighing 40 gm. or more is shown in Figure 11. The average of the check plots was 2.45 and of the high and

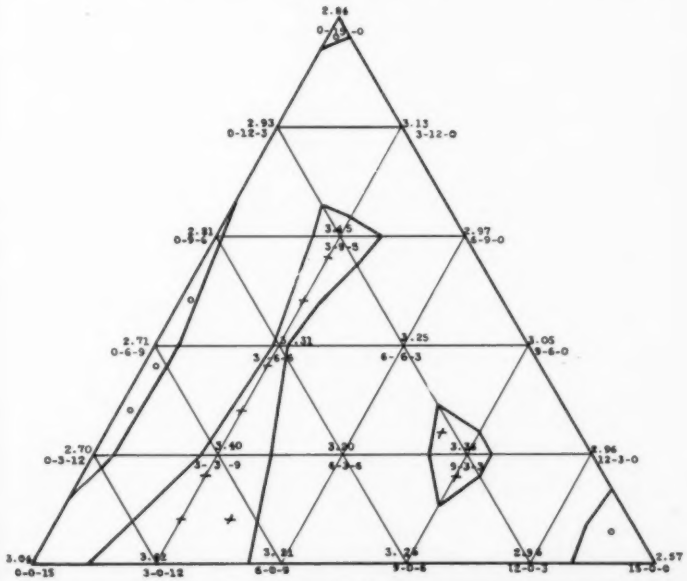


FIGURE 11.—Total number of potato tubers weighing 40 gm. or more when grown with different fertilizer mixtures. Areas of the greatest number are indicated by crosses, those of the lowest by circles

low yielding fertilizers 3.62 and 2.57, respectively. The five mixtures producing the greatest number of tubers were 3–0–12, 3–9–3, 3–3–9, 9–3–3, and 3–6–6, and those producing the fewest were 15–0–0, 0–3–12, 0–6–9, 0–9–6, and 0–15–0.

The average weight per tuber of the tubers weighing over 40 gm. is shown in Figure 12. The five highest yielding mixtures were 12–3–0, 9–3–3, 6–3–6, 3–0–12, and 9–0–6, and the lowest were 0–15–0, 0–3–12, 0–6–9, 0–9–6, and 3–12–0. The average weight of the tubers from the unfertilized checks was 71.96 gm., while the average weight of the tubers from the high and low plots of the fertilized series was 89.65 and 75.05 gm., respectively.

The total weight, number, and size of tubers over 40 gm. from the five high and five low-yielding fertilizers are shown in Table 10. It is seen that the five fertilizers producing highest yields carried both nitrogen and potash, while phosphoric acid was present in only

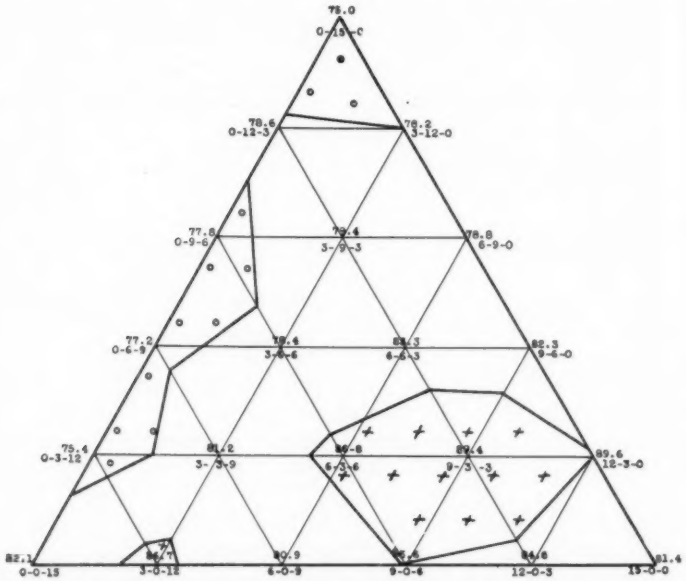


FIGURE 12.—Average weight of potato tubers weighing 40 gm. or more when grown with different fertilizer mixtures. Areas of the largest tubers are indicated by crosses, those of the smallest by circles

three. On the other hand, this element was present in four of the five low-yielding mixtures, while either nitrogen or potash or both were absent from all.

The fertilizer mixtures producing the largest number of tubers are characterized by medium to high nitrogen and potash. The average number of tubers per hill was 3.71 for the high and 2.73 for the low group of mixtures a difference of nearly 36 per cent in favor of the mixtures containing both nitrogen and potash. It is worthy of note that high potash content in the absence of nitrogen did not permit the development of a large number of tubers, and that low content of potash was compensated for by the presence of liberal quantities of nitrogen.

TABLE 10.—*Effect of fertilizer ratios upon the weight per hill, number of tubers, and weight per tuber of potatoes weighing 40 gm. or more*

High-yielding fertilizer mixtures	Yield per hill	Low-yielding fertilizer mixtures	Yield per hill	High-yielding fertilizer mixtures	Tubers
	Grams		*Grams*		*Number*
3-0-12	317.2	0-3-12	203.6	3-0-12	3.62
9-3-3	305.7	0-6-9	208.5	3-9-3	3.45
6-3-6	284.5	15-0-0	213.1	3-3-9	3.40
9-0-6	283.5	0-15 0	219.8	9-3-3	3.36
3-3-9	281.7	0-9-6	222.1	3-6-6	3.31

Low-yielding fertilizer mixtures	Tubers	High-yielding fertilizer mixtures	Weight per tuber	Low-yielding fertilizer mixtures	Weight per tuber
	Number		*Grams*		*Grams*
15-0-0	2.57	12-3-0	89.65	0-15-0	75.05
0-3-12	2.70	9-3-3	89.45	0-3-12	75.39
0-6-9	2.71	6-3-6	86.79	0-6-9	77.17
0-9-6	2.81	3-0-12	86.71	0-9-6	77.87
0-5-0	2.84	9-0-6	85.61	3-12-0	78.21

The mixtures producing largest tubers in this class are approximately the same as those producing largest total yields, and differ from those producing largest number of tubers mainly in their higher nitrogen and lower potash content. The mixtures producing smallest tubers are essentially the same as those producing fewest tubers and smallest total yield. Although heavy tubers in this class were produced by mixtures of high nitrogen content in the absence of potash, moderately low nitrogen was in part compensated for by high potash concentrations. Small tubers in this class were associated with mixtures containing no nitrogen, or those containing small quantities of nitrogen without adequate potash.

It is clear that high yields of marketable tubers resulted where both nitrogen and potash were present. Both constituents are important since abundant potash is required for the development of the individual tubers and the presence of nitrogen insures their reaching a marketable size.

TUBERS WEIGHING 75 GM. OR MORE

The influence of the different fertilizer mixtures on the total yield of tubers weighing over 75 gm. is shown in Table 11. Although the fertilizer experiment covered a 4-year period, so few potatoes weighing more than 75 gm. were produced in 1925 that this year was not included in the averages. The yields in 1926, 1927, and 1928 were more nearly normal, and the average for these years represents average conditions. The average yield of the checks was 123.8 gm. and of the high-yielding and low-yielding mixtures, 275.6 and 131.0 gm., respectively.

TABLE 11.—*Influence of different fertilizer mixtures on average number and weight per hill, and average weight of potato tubers weiging 75 gm. or more* [a]

Fertilizer mixture used	Average number of tubers per hill	Average weight per hill [b]	Average weight per tuber [c]	Fertilizer mixture used	Average number of tubers per hill	Average weight per hill [b]	Average weight per tuber [c]
		Grams	*Grams*			*Grams*	*Grams*
Check	1.21	123.78	100.6	0-3-12	1.33	130.99	98.6
0-15-0	1.56	163.33	103.7	3-3-9	2.10	224.04	106.7
0-12-3	1.78	183.41	103.2	6-3-6	2.23	251.60	112.1
3-12-0	1.87	197.54	104.6	9-3-3	2.42	275.64	113.4
0-9-6	1.50	148.93	98.6	12-3-0	2.11	245.65	115.1
3-9-3	2.09	223.42	106.2	0-0-15	1.94	208.62	107.3
6-9-0	1.81	195.56	106.5	3-0-12	2.48	272.80	109.7
0-6-9	1.38	137.04	99.1	6-0-9	1.93	210.11	108.7
3-6-6	1.79	191.43	106.5	9-0-6	2.10	236.04	111.5
6-6-3	2.22	243.74	108.6	12-0-3	1.76	191.15	107.4
9-6-0	2.03	226.47	110.2	15-0-0	1.47	159.01	104.7

[a] Derived from 3 yearly averages. [b] Probable error, 9.60 per cent. [c] Probable error, 1.74 per cent.

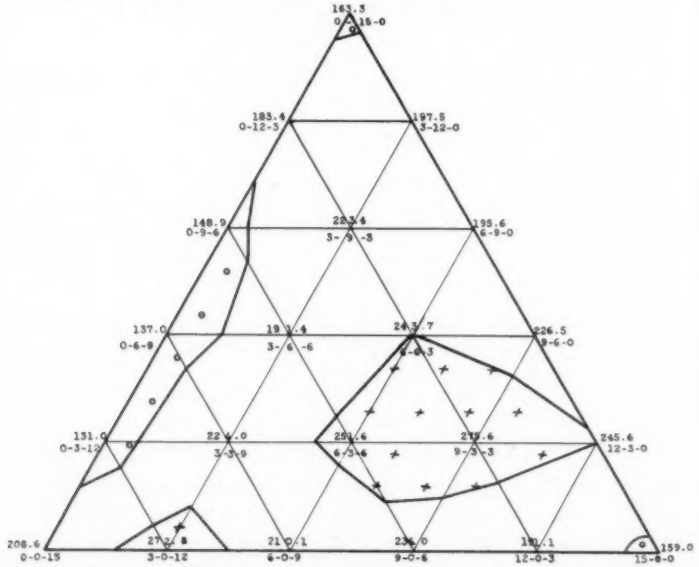


FIGURE 13.—Total yield of potato tubers weighing 75 gm. or more when grown with different fertilizer mixtures. Areas of the largest yields are indicated by crosses, those of the smallest by circles

The total yield of tubers weighing over 75 gm. is shown in Figure 13. The five most productive mixtures were 9–3–3, 3–0–12, 6–3–6, 12–3–0, and 6–6–3, and the five least productive, 0–3–12, 0–6–9, 0–9–6, 15–0–0, and 0–15–0. Here, as in the two other classes of tubers considered, highest yields were produced by fertilizer ratios medium to high in nitrogen and potash and low in phosphoric acid. On the other hand, a deficiency in nitrogen appears to be the most important factor in determining low yields in the case of the larger tubers.

The average number of tubers per hill is shown in Figure 14. On the check plots, 1.21 tubers weighing over 75 gm. were produced per hill; from the highest-yielding mixture the average was 2.48 per hill, while from the lowest-yielding the average was only 1.33. The five mixtures producing the largest number of tubers in this class were 3–0–12, 9–3–3, 6–3–6, 6–6–3, and 12–3–0, and those producing the smallest number were 0–3–12, 0–6–9, 15–0–0, 0–9–6, and 0–15–0.

The average weight of all tubers over 75 gm. is shown in Figure 15. The average for the checks was 100.6 gm. The five high-producing mixtures were 12–3–0, 9–3–3, 6–3–6, 9–0–6, and 9–6–0; and the low-producing ones were 0–3–12, 0–9–6, 0–6–9, 0–12–3, and 0–15–0.

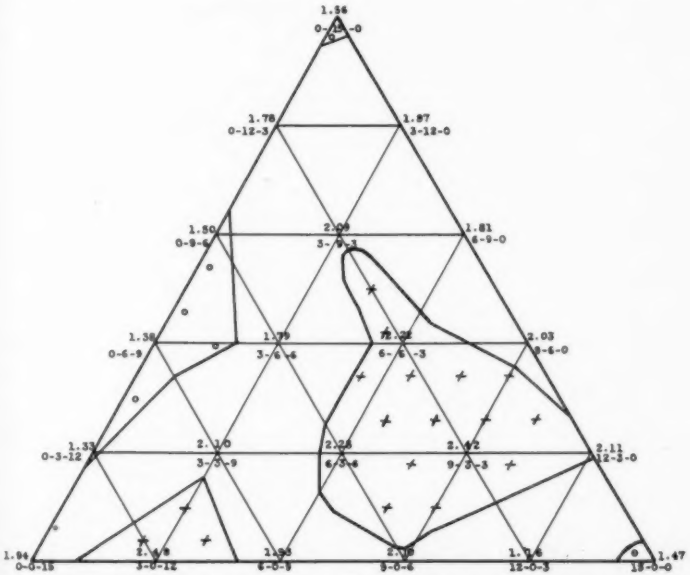


FIGURE 14.—Total number of potato tubers per hill weighing 75 gm. or more when grown with different fertilizer mixtures. Areas of the largest number of tubers are indicated by crosses, those of the smallest by circles

In Table 12 are shown the total yield, number, and weight, per tuber of potatoes, weighing over 75 gm., from both the high and low yielding mixtures. It will be seen that the five mixtures that produced the largest total yields also produced the greatest number of tubers. It will likewise be observed that three of these mixtures are included in the five that produced the largest tubers. In the 75-gm. class the average nitrogen and potash content of the five mixtures that produced the largest yields and largest total number of tubers is 7.2 and 4.8, respectively, as compared with 9 and 3 per cent, the average of the five mixtures that produced the largest tubers. The average phosphoric acid content of each of the three high groups is 3 per cent. It is apparent that a fertilizer mixture carrying medium to high nitrogen and potash with low to medium phosphoric acid

produced the largest number of tubers weighing more than 75 gm. and at the same time produced the largest average yields.

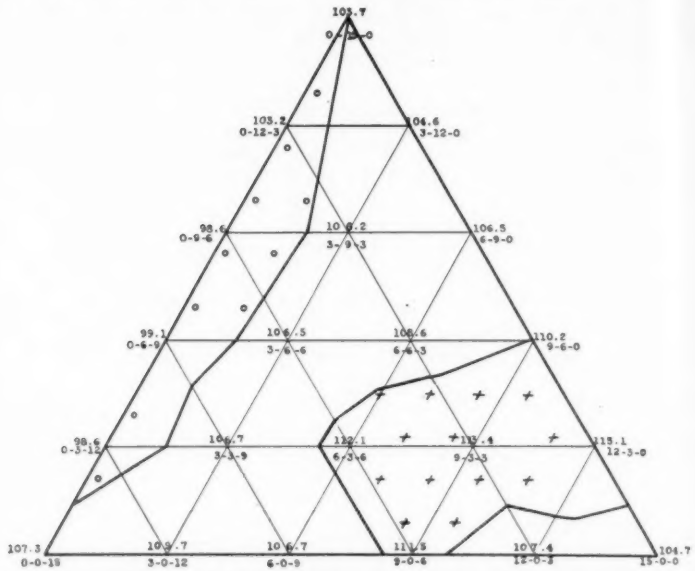


FIGURE 15.—Average weight of potato tubers weighing 75 gm. or more when grown with different fertilizer mixtures. Areas of the largest tubers are indicated by crosses, those of smallest by circles

TABLE 12.—*Effect of fertilizer mixtures upon the weight per hill, number of tubers, and weight per tuber of potatoes weighing 75 gm. or more*

High-yielding fertilizer mixtures	Yield per hill	Low-yielding fertilizer mixtures	Yield per hill	High-yielding fertilizer mixtures	Tubers
	Grams		*Grams*		*Number*
9-3-3	275.6	0-3-12	131.0	3-0-12	2.48
3-0-12	272.8	0-6-9	137.0	9-3-3	2.42
6-3-6	251.6	0-9-6	148.9	6-3-6	2.23
12-3-0	245.6	15-0-0	159.0	6-6-3	2.22
6-6-3	243.7	0-15-0	163.3	12-3-0	2.11
Average:					
7.2-3-4.8	257.8	3-6.6-5.4	147.8	7.2-3-4.8	2.29

Low-yielding fertilizer mixtures	Tubers	High-yielding fertilizer mixtures	Weight per tuber	Low-yielding fertilizer mixtures	Weight per tuber
	Number		*Grams*		*Grams*
0-3-12	1.33	12-3-0	115.1	0-3-12	98.6
0-6-9	1.38	9-3-3	113.4	0-9-6	98.6
15-0-0	1.47	6-3-6	112.1	0-6-9	99.1
0-9-6	1.50	9-0-6	111.5	0-12-3	103.2
0-15-0	1.56	9-6-0	110.2	0-15-0	103.7
3-6.6-5.4	1.45	9-3-3	112.2	0-9-6	100.6

Of the five fertilizers that produced the lowest weight per hill as well as the smallest number and weight of tubers, there is a very marked agreement in the various groups. Of the five mixtures, four

appear in each group and one in two of the three groups. In all three groups nitrogen, phosphoric acid, and potash vary from 0 to 15 per cent, and these three ingredients are present either singly or in combinations of either nitrogen and phosphoric acid or of phosphoric acid and potash. In no instance do nitrogen and potash appear in the same fertilizer mixture in the low series.

DISCUSSION

In considering the influence of the different fertilizer mixtures on weight per hill it will be seen from Figure 16 that there is a general agreement in the response to the fertilizer treatments in the case of all tubers, of those weighing over 40 gm., and of those weighing over 75 gm. The complete mixtures gave highest average yields, followed in turn by those containing nitrogen and potash, nitrogen and phosphoric acid, and phosphoric acid and potash. In the case of those mixtures carrying single ingredients, highest yields resulted from the use of potash, followed in order by phosphoric acid and nitrogen. It is apparent that, with but one exception (the 3–0–12 ratio), in those mixtures in which the phosphoric acid remained constant while the nitrogen and potash varied, the largest yields followed the use of the fertilizers higher in nitrogen. In considering, for example, the fertilizers carrying nitrogen and phosphoric acid alone it will be observed that as the nitrogen decreases from 12 to 3 per cent while the phosphoric acid increases to the same extent, the yields fall off. In the case of the fertilizers containing only phosphoric acid and potash still greater decreases result where the potash content of the mixtures is increased while the phosphoric acid content is decreased. Where nitrogen and potash were used in combination, however, the yields were higher than where either of these were used with phosphoric acid.

The influence of nitrogen on total yield per hill is further seen from a consideration of Figures 7, 10, and 13.

The five low-yielding fertilizer mixtures occupy practically the same region in the triangle in the case of all tubers and of those weighing over 40 and 75 gm. This region is characterized by low nitrogen, low to high phosphoric acid, and medium to high potash. On the other hand, the ratios producing high yields occupy an area in the triangle characterized by medium to high nitrogen with low to medium phosphoric acid and low to high potash.

The influence of the fertilizer mixtures on the weight of the tubers is shown in Figure 16. From a consideration of those carrying single ingredients it will be seen that in the case of all tubers, largest average yields were produced by nitrogen, followed by potash and phosphoric acid, whereas in the case of the tubers weighing more than 40 gm. and more than 75 gm. largest yields were produced by the fertilizers made up entirely of potash, followed in order by the mixtures containing nitrogen and phosphoric acid.

From a consideration of the mixtures containing two or three ingredients it is clear that nitrogen is important in determining tuber size. In every case where the phosphoric acid content of the fertilizer was kept constant and the nitrogen and potassium content was varied, the largest tubers were produced by the mixtures having the

73318—31——5

higher nitrogen content. The same condition exists, with the exception of one mixture (3–0–12) in the case of the complete mixtures and those containing nitrogen and potash only.

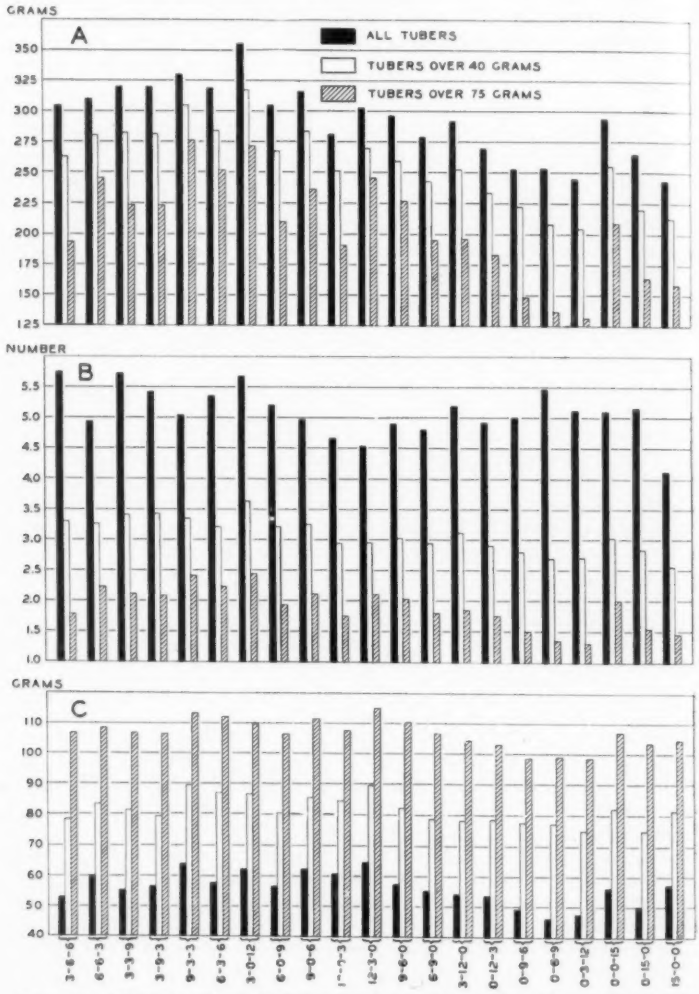


FIGURE 16.—Influence of different fertilizer mixtures on yield (A), number (B), and size (C) of all potato tubers, of tubers weighing 40 gm. or more and of those weighing 75 gm. or more

From a consideration of Figures 9, 12, and 15 it will be seen that the region in the triangle producing the smallest tubers is practically identical for each of the three classes of tubers. This region is characterized by no nitrogen, medium to high phosphoric acid, and medium to high potash. On the other hand, the region producing

largest tubers is characterized by high nitrogen with low phosphoric acid and potash.

The high average weight of the tubers in the 75-gm. class is due largely to increases in length. In the case of the five high-yielding mixtures, characterized by high nitrogen with none to medium potash, the tubers averaged 3 per cent wider and thicker than those from the five low-yielding mixtures, characterized by no nitrogen with medium to high potash. On the other hand, the tubers from the five high-yielding mixtures averaged 7.45 per cent longer than those from the low-yielding mixtures.

The data showing the influence of the fertilizer mixture on number of tubers for each of the three classes are presented in Figure 16. In the case of the mixtures carrying a single ingredient, the largest number of all tubers was produced by phosphoric acid, followed by potash and nitrogen, while in the case of the larger tubers, the greatest numbers were produced by potash, followed in order by phosphoric acid and nitrogen.

In the case of all tubers, the largest numbers were produced by mixtures high in potash and low in nitrogen, but the reverse was true in the case of the larger tubers. This was so of both the complete and incomplete mixtures. The 3–6–6 mixture, for example, produced 5.74 and 1.79 tubers per hill in the case of all tubers and of those weighing over 75 gm., respectively. On the other hand, the 6–6–3 mixture produced 4.96 and 2.22 tubers per hill for these two classes. The influence of fertilizer mixtures on number of tubers is clearly seen from an examination of figures 8, 11, and 14. In the case of all tubers the five mixtures producing the smallest number are characterized by medium to high nitrogen with little or no potash. The five high-yielding mixtures, on the other hand, occupy a region of no nitrogen to medium nitrogen with medium to high potash. The phosphoric acid range is identical in both the high and low regions.

When the number of tubers weighing more than 40 gm. is considered (fig. 11) it is apparent that the high region and the low region, particularly the latter, have shifted considerably; this now occupies, in part, the region formerly occupied by the five mixtures producing largest number of all tubers. When the tubers weighing more than 75 gm. are considered (fig. 14), the high region is seen to have shifted still farther in the direction of higher nitrogen, while the five low-yielding mixtures occupy the part of the triangle identified with low nitrogen and high potash. It is clear that while potash is important in the production of tubers, nitrogen is more important in determining the size of the individual tuber. This agrees in part with the finding of Bushnell (3). He states that nitrogen increased the number of tubers per plant, but that potash had no constant influence on number. However, Bushnell included only those tubers larger than 1¼ inches in his counts; had he included all tubers his results might have been more nearly in accord with those presented here.

It is worthy of note that the effect of the fertilizer mixtures on yield of the heavier class of tubers has been accomplished primarily through the effect on number of tubers of the larger sizes and to a very minor extent by changing the average weight of such tubers. Thus the difference between the least productive and the most productive fertilizers was 74.5 per cent for total yield of the heavy class

of tubers, 57.9 per cent for the total number of such tubers, and only 11.8 per cent for their average weight. Although increasing the percentage of nitrogen resulted in a slight increase in average weight, in general the weight of the tubers was nearly constant and yields were determined largely by the number of tubers of the heavier class.

The total number of all tubers was practically the same for the five fertilizer mixtures producing the greatest total yield of tubers weighing 75 gm. or over and for the five mixtures producing the lowest yields of this class of tubers. The difference between the two groups of mixtures in the case of all tubers was less than 3 per cent, as compared with 57.9 per cent when only the number of tubers of the heavier class are considered. Largest total yields of heavy tubers were, therefore, not caused by increasing the total number of all tubers formed but by increasing the percentage of tubers in the 75-gm. class. Three of the five fertilizer mixtures giving largest total yields also had the largest of tubers falling in the heavy class, and likewise all five of the least productive mixtures had the lowest number of tubers attaining a weight of 75 gm. or over.

It is also worth noting that only one of the fertilizer mixtures which produced the greatest number of all tubers was identical with those producing the greatest number of tubers in the group weighing 75 gm. or over. There was some correlation between the mixtures producing the greatest number of tubers weighing 40 gm. or over and those producing the greatest number weighing 75 gm. or over, but the correlation was far from perfect. On the other hand, the total yield of all tubers was influenced by the fertilizer mixtures in a manner quite similar to that of the yield of tubers in the two heavier groups.

SUMMARY

In a series of experiments extending over a period of four years comparisons were made of 21 different fertilizer mixtures each containing 15 units of plant food. One hundred hill samples proved to be a satisfactory measure of the yield of the different plots.

The total yield as well as the number and weight of the tubers was influenced by seasonal conditions. Yields were influenced to a greater extent by differences in the number of tubers than by the weight of the individual tubers.

The longest tubers were produced in the season of greatest rainfall, but this is believed to have been due to the influence of the soil moisture content on the availability of the fertilizer rather than to its direct action on the tuber itself.

The fertilizer mixtures that produced the widest potatoes were characterized by low to high nitrogen, low phosphoric acid, and low to high potash. The thickest potatoes were produced by mixtures with low to high nitrogen, low phosphoric acid, and medium to high potash. Both thinner and narrower tubers were produced by mixtures made up of no nitrogen to low nitrogen and potash with high phosphoric acid. The longest potatoes were produced by mixtures medium to high in nitrogen and phosphoric acid and low in potash, while the shortest tubers were produced by mixtures with no nitrogen to low nitrogen, low to medium phosphoric acid, and medium to

high potash. Tubers with highest shape index values were produced by mixtures very similar to those that produced the longest tubers.

The quantity of potassium present appears to be chiefly responsible for modification of the shape of the tuber. When fertilizer mixtures deficient in potassium but containing nitrogen were used, tubers with high index values were obtained, but when nitrogen was absent and potassium was abundant, the tubers were shorter, i. e., the index values were lower.

When all tubers are considered, the largest are found to have been produced by mixtures carrying large amounts of nitrogen with none to small amounts of phosphoric acid and none to large amounts of potash. The greatest number of tubers in this class were produced by mixtures low in nitrogen, low to high in phosphoric acid, and high in potash, while mixtures high in nitrogen with none to little potash produced fewest tubers. Largest total yields of tubers in this class were produced by mixtures having both nitrogen and potassium in medium to large amounts, the phosphoric acid being present in small amounts.

Of the tubers weighing 40 gm. or over, the largest were produced by mixtures characterized by medium to high nitrogen, low phosphoric acid, and low to high potash. The fewest tubers were produced by mixtures characterized by none to low nitrogen, high phosphoric acid, and low to high potash. Highest total yields of tubers in the 40-gm. class were produced by mixtures containing medium to high nitrogen and potash and low phosphoric acid.

Of the tubers weighing 75 gm. or more, the largest were produced by mixtures high in nitrogen, low to medium in phosphoric acid, and low to medium in potash. The largest number of tubers in this class was produced by mixtures medium to high in nitrogen and potash, while mixtures containing no nitrogen produced fewest tubers. Largest total yields of tubers in the 75-gm. class were produced by mixtures carrying medium to high nitrogen, with no potash to high potash, and no phosphoric acid to medium phosphoric acid. Low total yields, on the other hand, were produced by mixtures deficient in either nitrogen or potash. In general, however, the absence of nitrogen was the most important factor contributing to low yields. The influence of the fertilizer mixtures on total yield of tubers weighing 75 gm. or more was due to the production of a greater number of tubers in this class rather than to an increase in the size of the individual tubers.

LITERATURE CITED

(1) ANONYMOUS.
1908. THE PROBABLE ERROR OF A MEAN. By Student. Biometrika 6: 1–25, illus.

(2) ———
1917. TABLES FOR ESTIMATING THE PROBABILITY THAT THE MEAN OF A UNIQUE SAMPLE OF OBSERVATION LIES BETWEEN — ∞ AND ANY GIVEN DISTANCE OF THE MEAN POPULATION FROM WHICH THE SAMPLE IS DRAWN. By Student. Biometrika 11: 414–417.

(3) BUSHNELL, J.
1924. EFFECT OF FERTILIZERS ON NUMBER AND SIZE OF POTATO TUBERS. Potato Assoc. Amer. Proc. 11: 108–113.

(4) CLARK, C. F.
1921. DEVELOPMENT OF TUBERS IN THE POTATO. U. S. Dept. Agr. Bul. 958, 27 p., illus.

(5) HARDENBURG, E. V.
1923. ECOLOGICAL FACTORS AFFECTING TUBER-SET IN POTATOES. Potato Assoc. Amer. Proc. 10: 165–172.
(6) ———
1926. SOME FACTORS AFFECTING TUBER SHAPE IN POTATOES. Potato Assoc. Amer. Proc. 13: 42–45.
(7) KRANTZ, F. A.
1923. PERMANENCE OF VARIETY IN THE POTATO. Jour. Agr. Research 23: 947–962, illus.
(8) NEUMAN, H.
1925. DIE BEEINFLUSSUNG DER KARTOFFELKNOLLE DURCH AUSSERE VERHÄLTNISSE. Jour. Landw. 73: [7]–38.
(9) ROBBINS, W. R., NIGHTINGALE, G. T., SCHERMERHORN, L. G., and BLAKE, M. A.
1929. POTASSIUM IN RELATION TO THE SHAPE OF THE SWEETPOTATO. Science (n. s.) 70: 558.
(10) SCHERMERHORN, L. G.
1924. SWEET POTATO STUDIES IN NEW JERSEY. N. J. Agr. Expt. Sta. Bul. 398, 19 p., illus.
(11) SCHREINER, O., and SKINNER, J. J.
1918. THE TRIANGLE SYSTEM FOR FERTILIZER EXPERIMENTS. Jour. Amer. Soc. Agron. 10: 225–246, illus.
(12) WERNER, H. O.
1924. RELATION OF ENVIRONMENT TO SPINDLE-TUBER SYMPTOMS. Potato Assoc. Amer. Proc. 11: 102–106.

RELATIVE NUMBERS OF TWO SPECIES OF RHIZOBIUM IN SOILS[1]

By J. K. WILSON

Professor of Soil Technology, New York (Cornell) Agricultural Experiment Station

INTRODUCTION

The legume bacteria, like other soil organisms, exist in the soil in equilibrium with many complex conditions, and they vary in number according to the variations in any one or more of these conditions. A variation in a certain condition may increase the number of one species of organisms and at the same time may decrease the number of other organisms. The legume bacteria, however, differ from many other soil organisms in that they are unable to attack simple insoluble carbohydrates, such as starch, and in consequence are very largely, if not entirely, dependent on the activities of other species to furnish them with carbohydrates. In consequence of this, the legume bacteria probably are influenced by a greater number of conditions, or are influenced to a greater degree, by variations in a given set of conditions, than are most soil organisms.

Certain species, however, may be able to utilize a definite quantity of energy to better advantage than others, or to compete more successfully with others, for a given available supply of energy, or to exist more easily in the presence either of other organisms or of certain soluble salts, such as calcium sulphate. If this be true, these species should be more numerous than others under most conditions. Evidence that such is the case with repect to *Rhizobium trifolii* as compared with *R. leguminosarum* has already been presented[2]; a study of the relative numbers throughout the season of the two species in samples of Dunkirk silty clay loam under different fertilizer treatments showed that the ratio of the former to the latter species varied between 1.33:1 and 5:1. Further evidence that *R. trifolii* is generally more numerous than *R. leguminosarum* is presented in this paper.

METHODS

The method used in making this study was essentially the same as that followed in the work just referred to above. Samples were taken from 1/100-acre plots that had been designed for other studies but which were suitable for this work. The samples consisted of 10 to 12 small takings from the first 3 to 4 inches of soil. These were mixed on paper to insure uniformity, and a moisture determination was then made. While this was being done the samples were kept at a temperature of about 10° C. The reaction of the composite sample of soil was determined by the quinhydrone method.

To avoid variations that might occur in small samples and to aid in obtaining uniformity of results, enough moist sample was taken to give 100 gm. of dry soil. This was added to water and dispersed three minutes with a mechanical agitator. During this time enough

[1] Received for publication Feb. 18, 1931; issued August, 1931.

[2] WILSON, J. K., SEASONAL VARIATION IN THE NUMBER OF TWO SPECIES OF RHIZOBIUM IN SOIL. Soil Sci. 30: 289–296, 1930.

water was added to make a total volume of 1,000 c. c. Dilutions were made from this muddy suspension. Throughout the work care was exercised to avoid contamination with the legume organism.

A portion of the suspension equivalent to a certain quantity of soil was put on the surface of sterile soil in tumblers where the host plants were to be grown. Since both *Rhizobium trifolii* and *R. leguminosarum* may be present in the same dilution it was possible to grow both host plants in association in the same tumbler and thus determine the presence or absence of both species on the dilution by observing the production of nodules. This procedure was satisfactory because both *Trifolium pratense* (red clover) and *Vicia villosa* (vetch) showed nodulation within 14 days after planting in case the proper organisms were present in the dilution.

The tumblers, which held about 200 gm. of sandy soil that was known to be suitable for the growth of the two organisms and for nodulation on the two host plants, were kept covered with paper which was held in place by a rubber band not only during sterilization and subsequent handling but also until the plantlets were in need of light. If contamination occurred after the paper was removed it did not have time to produce nodules and change the results. Seeding was delayed for five to six days after the soils received the inoculating suspension in order to permit the establishment of a high legume bacteria content in case the organism was introduced. Heavy seeding was practiced in order to insure an adequate number of plants for inspection and to increase the chances for nodulation. Before the seeds were planted they were treated either with a solution of calcium hypochlorite or with concentrated sulphuric acid or sometimes with both to free them of any adhering legume organisms. After the treated seeds were spread on the surface of the soil in the tumblers they were covered with sterile soil.

The first studies which indicated that soils may support a larger number of *Rhizobium trifolii* than of *R. leguminosarum* were made in 1926. Since then additional studies have been made. These later studies indicated the number of each species that might be expected in the soils and made possible a knowledge of the spread of dilutions necessary to determine fairly accurately the number of each species per gram of soil. The results of such studies are recorded in this report.

THE RELATIVE NUMBERS OF TWO SPECIES OF RHIZOBIUM IN DUNKIRK GRAVELLY LOAM SOIL

TABLE 1.—*The relative numbers of Rhizobium trifolii and R. leguminosarum in Dunkirk gravelly loam soil at Geneva, N. Y., August 5, 1930*

Plot No.	Annual treatment per acre for 40 years	pH	Moisture in sample	Number per gram of—	
				R. trifolii	R. leguminosarum
	Pounds		*Per cent*		
2	1,000 Na_2SO_4[a]	6.3	4.9	25,000	10,000
3	Untreated	6.0	5.4	10,000	5,000
4	2,000 $MgSO_4$	6.0	5.2	50,000	100,000
5	Untreated	6.1	4.7	25,000	10,000
6	2,000 $CaSO_4$	6.6	5.3	1,000,000	10,000
8	1,200 $FeSO_4$	5.3	4.5	5,000	2,500
9	Untreated	6.4	5.2	50,000	25,000
10	500 NaCl[b]	7.0	5.2	10,000	2,500
11	Untreated	7.5	5.0	100,000	10,000
12	2,000 $CaCO_3$	8.2	7.4	10,000	5,000

[a] Last five years increased to 1,600 pounds annually.
[b] Last five years increased to 600 pounds annually.

Table 1 gives data obtained from soil samples taken at Geneva, N. Y., on August 5, 1930. The soil is classed as Dunkirk gravelly loam. The plots from which these samples were taken were either fertilized annually for 40 years or untreated, as indicated in the table All plots were growing alfalfa successfully when samples were taken and had grown this crop for several years previously.

The outstanding fact brought out by a study of Table 1 is that in only one case, that of plot 4, which had received 2,000 pounds of magnesium sulphate annually, is the number of *Rhizobium trifolii* exceeded by that of *R. leguminosarum*. In the other 9 cases the number of *R. trifolii* is larger, the aggregate showing a ratio of over 15 : 1. Even the alkaline plots—Nos. 10, 11, and 12—show a larger number of *R. trifolii*. The high count of *R. trifolii* found in plot 6 where calcium sulphate was applied is in agreement with the work of Pitz [3], who found that this salt when used in pure-culture studies stimulated the growth of this organism.

THE RELATIVE NUMBERS OF TWO SPECIES OF RHIZOBIUM IN CHENANGO GRAVELLY LOAM SOIL AND IN WOOSTER SANDY LOAM SOIL

Samples were collected at Warsaw, N. Y., on June 27, 1930, from plots located on two soil types. Although these plots, which were about one-hundredth of an acre in size, have been used quite largely for potato crops, oats were growing on them at the time of sampling.

All the plots had received a basic treatment of superphosphate at the rate of 150 pounds to the acre in the spring of 1929. Certain other treatments which were designed to change the soil reaction are shown in Table 2. It will be noted that samples from all plots were distinctly acid, the difference in acidity in no instance exceeding one pH unit. The samples contained about 10 per cent moisture. The data from both soil types show that they were more efficient in supporting *Rhizobium trifolii* than they were were in supporting *R. leguminosarum*. The Chenango gravelly loam gave a ratio of the former to the latter of 7.5: 1 and the Wooster sandy loam a ratio of at least 77.3: 1. Other determinations in these soils made from samples taken in September, 1930, showed about the same relationship.

TABLE 2—*Relative numbers of Rhizobium trifolii and R. leguminosarum in Chenango gravelly loam and in Wooster sandy loam soil at Warsaw, N. Y., June 27, 1930*

[All plots given a basic treatment of 150 pounds superphosphate per acre, spring of 1929]

Plot No.	Treatment	Soil pH and number of bacteria per gram of—					
		Chenango gravelly loam			Wooster sandy loam		
		pH	R. trifolii	R. leguminosarum	pH	R. trifolii	R. leguminosarum
1	Untreated	5.8	[a] 10,000	[a] 10,000	5.6	[a] 10,000	500
2	50 pounds sulphur	5.5	[a] 10,000	2,500	5.5	[a] 10,000	100
3	100 pounds sulphur	5.5	[a] 10,000	2,500	5.8	[a] 10,000	10
4	200 pounds sulphur	4.8	2,500	100	5.5	[a] 10,000	10
5	Untreated	5.3	10,000	1	5.8	[a] 10,000	100
6	50 pounds sulphur	5.2	5,000	500	5.6	500	[b]
7	100 pounds sulphur	5.0	5,000	1,000	5.6	5,000	10
8	200 pounds sulphur	5.3	5,000	1,000	5.0	500	[b]
9	Untreated	5.4	10,000	50	5.5	500	[b]

[a] Larger than indicated.

[b] Less than 10 per gram.

[3] PITZ, W. EFFECT OF ELEMENTAL SULPHUR AND CALCIUM SULPHATE ON CERTAIN OF THE HIGHER AND LOWER FORMS OF PLANT LIFE. Jour. Agr. Research 5 : 771-780, illus. 1916.

THE RELATIVE NUMBERS OF RHIZOBIUM IN TWO SPECIES OF DUNKIRK SILTY LOAM

Data obtained from an analysis of soil samples collected from Caldwell field at Ithaca, N. Y., on March 19, 1929, are presented in Table 3. They show the capacity of Dunkirk silty clay loam soil to support *Rhizobium trifolii* and *R. leguminosarum*. Sixteen plots that had received various soil amendments were examined. In 1920 and again in 1925 all these plots were manured at the rate of 10 tons to the acre. Plots 1 to 7, inclusive, and 10, 13, and 16 received no other treatment. The other plots were treated as indicated.

Pisum arvense (garden pea), the host plant for *Rhizobium leguminosarum*, was grown on plots 5 and 11 in 1921 and *Trifolium pratense*, the host plant for *R. trifolii*, was grown on them in 1923. For three years before the samples were taken grasses were grown on all the plots but no attempt was made to keep out volunteer species of Trifolium. The moisture content of all samples was slightly above 20 per cent.

TABLE 3.—*Relative members of Rhizobium trifolii and R. leguminosarum in Dunkirk silty clay loam soil at Ithaca, N. Y., March 19, 1929*

Plot No.	Treatment to the acre	Soil pH	Number of bacteria per gram of soil	
			R. trifolii	R. leguminosarum
1	10 tons manure in 1920 and in 1925	5.2	10,000	[a]
2	do	5.8	25,000	[b] 100
3	do	5.7	50,000	[a]
4	do	5.7	25,000	[b] 100
5	do	5.5	50,000	5,000
6	do	5.5	500,000	[b] 100
7	do	5.6	50,000	100
8	4,000 pounds Ca $(OH)_2$ in 1920 and in 1921	7.1	500,000	1,000
9	do	7.6	250,000	1,000
10	10 tons manure in 1920 and in 1925	6.5	100,000	100
11	4,000 pounds Ca $(OH)_2$ in 1920 and in 1921	8.0	[c] 500,000	[c] 50,000
12	do	7.9	[c] 500,000	500
13	10 tons manure in 1920 and in 1925	6.6	[c] 500,000	250
14	200 pounds K_2SO_4 1912 to 1924, inclusive	5.7	[c] 500,000	100
15	200 pounds K_2SO_4 1912 to 1924, inclusive, and 4,000 pounds Ca$(OH)_2$ in 1920 and in 1921	7.3	[c] 500,000	100
16	10 tons manure in 1920 and in 1925	6.4	250,000	10

[a] Less than 1. [b] Less than indicated. [c] More than indicated.

The figures from these tests show rather strikingly that this Dunkirk silty clay loam supported a larger number of *Rhizobium trifolii* than of *R. leguminosarum*. This was true even though the soil had received various types of amendments, some of which had changed the soil reaction. The number of *R. leguminosarum* in the soil of plot 11 was probably not very much larger than that given in the table, for a test made one month later showed that the soil contained between 10,000 and 25,000 organisms. There seems to be no relation between the number of *R. trifolii* in the soil and the frequency of its host plant on the soil.

Plots 6 and 12 were kept bare of all vegetation from 1921 to 1924, inclusive, timothy was grown from 1925 to 1928. However, in spite of the fact that the host plant was absent from these plots for seven years, the organisms were probably as numerous or even more

numerous than when some species of Trifolium formed a small portion of the vegetation. The two plots which grew *Pisum arvense* in 1921 showed the largest number of *Rhizobium leguminosarum* when examined in 1929. It is doubtful, however, in the light of what is known about the seasonal variation of this organism whether these figures have any significance.

DISCUSSION

From the data presented it is apparent that the four soil types examined support *Rhizobium trifolii* more effectively than they do *R. leguminosarum*. Whether this is due to moisture conditions is doubtful. Ten samples from a Dunkirk gravelly loam soil containing 5 per cent moisture, 9 from a Chenango gravelly loam soil and 9 from a Wooster sandy loam soil containing 10 per cent moisture, and 16 from a Dunkirk silty clay loam soil containing 20 per cent moisture show the same tendency. In 42 of the 44 samples examined this relationship existed. In one of the two cases which did not show this relationship the plot had received a large yearly application of magnesium sulphate, and in the other case the data obtained were insufficient to justify a comparison. Neither the acidity nor the alkalinity of the soil appears to have had anything to do with this relationship. It was found to exist in soil as acid as pH 4.5 and in soil as alkaline as 8.2. It is evident, moreover, that seasonal variation has no part in this, for samples collected on March 19, on June 27, and on August 5 exhibited similar ratios. Samples collected in September, the data from which are not presented in this paper, confirmed these findings. Since these 44 samples were taken from soils that had not produced a crop of either host plant for several years no direct evidence is available to show that this relationship would have been modified if the plots had grown the host plants the same season in which the samples were taken.

The only evidence obtained in this study which might be regarded as furnishing a possible explanation of this relationship is that from plots to which certain sulphates had been applied. One plot on Dunkirk gravelly loam soil that had received annually for 40 years 2,000 pounds of calcium sulphate per acre seemed to favor the existence of *Rhizobium trifolii*. This plot gave a higher count of this organism than any other of the 10 plots on this soil. It also gave a higher count than most of the plots examined. Pitz[4] found that calcium sulphate used in pure-culture studies stimulated the growth of this organism, whil . iller[5] reported that it increased root development and the number of nodules on clover. The writer did not observe this effect, however, where the sulphates of potassium, sodium, magnesium, and iron (ferrous) had been applied. Another plot on this same soil that had received 2,000 pounds per acre of magnesium sulphate annually for 40 years seemed to favor the existence of *R. leguminosarum*. Of the 43 plots examined from which there were sufficient data for comparison, this is the only plot on which this organism was present in larger numbers than *R. trifolii*.

On the basis of these observations it might be suggested that the type of inorganic salts present in the soil solution may be partly responsible for the variability of soils in their capacity to support

[4] PITZ, W. Op. cit.

[5] MILLER, H. G. RELATION OF SULPHATES TO PLANT GROWTH AND COMPOSITION. Jour. Agr. Research 17: 87–102, illus. 1919.

bacteria of the same genus. It is possible that other agents such as the quality of the organic matter may also be important.

SUMMARY

Soil samples were collected from four different soil types. Plots of each soil type had received various soil amendments, some annually for 40 years. A total of 44 samples was taken, and the number of *Rhizobium trifolii* and *R. leguminosarum* in each was found. The criterion by which the numbers of the two species were determined was nodule production, which was brought about from suspensions representing definite portions of soil. Since the numbers of *R. trifolii* and *R. leguminosarum* were determined not only in the same samples of soil but also in the same fraction of a gram a comparison of the two species was possible.

Soils in general were found to support a larger number of *Rhizobium trifolii* than of *R. leguminosarum*. Forty-two of the 44 samples taken from four soil types showed this relationship.

This difference in the capacity of soils to maintain the population of one organism at a higher level than that of another of the same genus does not seem to be related to soil reaction, to moisture content, or to seasonal variation. There were some indications that it might be associated with the type of inorganic salts present in the soil.

THE FEEDING HABITS OF SOME LEAF HOPPERS OF THE GENUS EMPOASCA [1]

By FLOYD F. SMITH, *Associate Entomologist*, and F. W. POOS, *Senior Entomologist*, *Division of Cereal and Forage Insects, Bureau of Entomology, United States Department of Agriculture* [2]

INTRODUCTION

From the collections of Empoasca [3] made during 1929 and 1930, in the course of studies on leaf-hopper injury to legumes, several different species were isolated. The life histories and habits of these species are being studied, and the types of injury that they occasion on economic plants. This paper reports a study of the external symptoms produced on the plant, and of the particular tissues fed upon, by six species of Empoasca. The habits of these species as they may be correlated with their physiology, are discussed. Since this type of work involves a considerable knowledge of plant cytology and plant physiology, the writers have attempted to describe actual observations, and to discuss only in a limited way the theories and conclusions concerning the nature of the injury caused by *E. fabae* which have been advanced by previous workers. Only those references to previous work which seem to have a direct bearing on the problem have been cited in this paper. The present study was intended primarily as a foundation for considerable future work which now seems necessary in order to solve satisfactorily the problem of determining the exact cause of the injury produced by these species of Empoasca.

TECHNIC

Individuals used in this work were taken from pure lines of each species. After it had been determined that individuals of the two sexes were similar in their feeding habits and in the symptoms produced, males were selected for producing injury. Their specific identity was later determined by examination of the internal genitalia. The work was carried on in the greenhouse at a temperature of 75° to 80° F. Individuals of each species, except *maligna*, which was studied only on leaves of apple, were confined to the lower or upper surface of leaflets, to petioles, or to stems of alfalfa (*Medicago sativa*) in small cages.[4] Potato (*Solanum tuberosum*), Ladino clover (*Trifolium repens latum*), red clover (*T. pratense*), goldenrod (*Solidago* sp.), cowpea (*Vigna sinensis*), garden beans (*Phaseolus vulgaris*), and Jerusalem artichoke (*Helianthus tuberosus*) were also used as hosts. The behavior of the leaf hoppers and the effect upon the plants were recorded. Except in a few instances where partially wilted tissue was restored to turgidity by soaking in distilled water, the external

[1] Received for publication Feb. 11, 1931; issued August, 1931. This paper reports the results of one phase of a cooperative study of leaf-hopper injury to legumes made by the Division of Forage Crops and Diseases, Bureau of Plant Industry, and the Division of Cereal and Forage Insects, Bureau of Entomology, U. S. Department of Agriculture.

[2] The authors are grateful to Dr. D. M. DeLong, Ohio State University, for verifying the identity of the species of Empoasca included in this study.

[3] Order Homoptera, family Cicadellidae.

[4] For a description of this cage see: SMITH, FLOYD F. A NEW TYPE OF INSECT CAGE. Jour. Econ. Ent. 24: 14-16, illus. 1931.

and internal injury produced by each species was studied before the breaking down of the plant tissues, a condition which is accompanied by the later appearing symptoms, such as hopperburn of potato, yellowing of alfalfa, etc.

The material for cytological study, after exposure to the individual leaf hopper, was killed and fixed in one of the following agents: Formalin-alcohol-acetic-acid solution, weaker Flemming's fluid, chromo-acetic solution, Carnoy's fluid, or 95 per cent alcohol. The paraffin method was used for sectioning. Delafield's haematoxylin with eosin or with safranin was used in the early part of the work but later Flemming's triple stain (safranin, gentian violet, orange G) or safranin and light green, were used almost entirely.

TYPES OF INJURY

The species of Empoasca studied may be divided into two groups according to the type of injury produced.

GROUP 1.—PRIMARILY MESOPHYLL FEEDERS

This group includes five species, *bifurcata* DeL., *erigeron* DeL., *filamenta* DeL., *abrupta* DeL., and *maligna* Walsh. These leaf hoppers in feeding produce a definite stippling on the upper surface of the leaves, which is generally characteristic for each species. The stippling of *maligna* is very finely peppered: *erigeron* normally produces a slightly coarser type of stippling: and *bifurcata*, *filamenta*, and *abrupta* normally produce increasingly coarser stippling in the order named. In the feeding tests on alfalfa with *bifurcata*, *erigeron*, *filamenta*, and *abrupta* the amount of stippling produced in a given time by one individual increased in the order of the species named. (Fig. 1.) Since alfalfa is not the preferred host of *erigeron* or *bifurcata* the smaller amount and variation in type of stippled area produced by these species on this plant as compared with that of *filamenta* is probably to be expected. The feeding of *erigeron* and *bifurcata* on Solidago (figs. 2, 3), one of the preferred hosts, was much more extensive, which would also indicate that alfalfa is not the preferred host of these species. However, the type of feeding was typical for each of these species on all hosts tested. Usually adults of group 1, when confined to the leaves of alfalfa, lived until the greater part of the available leaf area had become stippled. One adult of *E. filamenta* lived for five days on a leaf area 15 mm. in diameter, while adults of *E. bifurcata* and *E. erigeron* have lived three and four days, respectively, on leaf tissue of the same area without being transferred to a fresh leaf.

In normal feeding, the leaf hopper evidently punctures the lower epidermis of the leaf and then feeds on the mesophyll tissue in all directions from this point, thus producing whitish spots (stippling) on the upper surface. The species of this group when confined to the upper surface of the leaf cause slightly smaller stippled areas than when confined to the lower surface, but apparently they are able to live as well on one surface as on the other. Often the center of the spot where the leaf hopper has fed appears green and normal. (Fig. 3.) If feeding punctures are made close together the stippled areas coalesce. When confined on the stems, petioles, or midveins of leaflets, the species of this group fed only for a few hours and died, usually

within 24 hours. Adults of *filamenta* and *abrupta* were apparently able to live slightly longer than *bifurcata* and *erigeron* on these plant parts. No wilting of the plant occurred beyond the point at which these species fed.

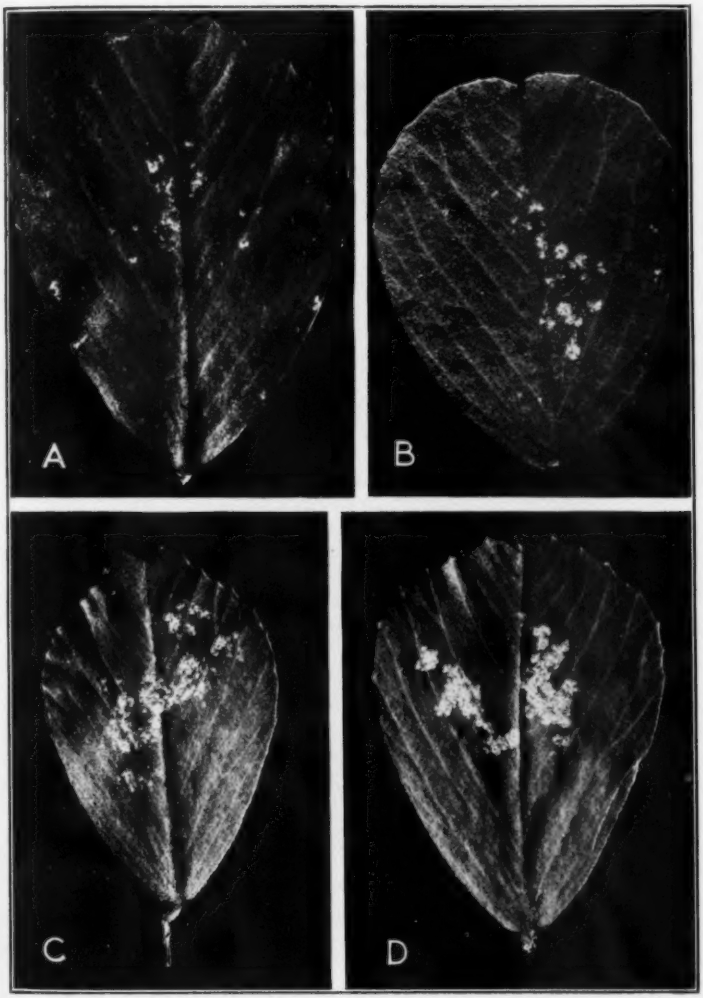


FIGURE 1.—Stippling produced on alfalfa leaflets by leaf hoppers of the genus Empoasca during 24 hours of feeding: A, By one adult *E. bifurcata*, ×3; B, by one adult *E. erigeron*, ×4; C, by one adult *E. filamenta*, ×3; D, by one adult of *E. abrupta*, ×3

A microscopic study of stained sections through the stippled areas on the leaves showed that the cell walls of the spongy mesophyll and of the palisade cells had been torn and the entire cell contents removed. In many instances large parts of the cell wall had disappeared. The

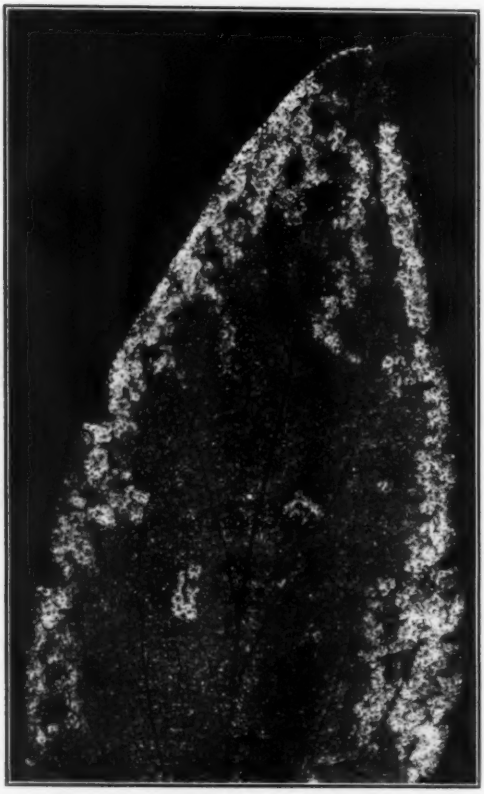


FIGURE 2.—Typical stippling produced by *E. erigeron* on *Solidago* sp., one of its preferred hosts. Compare with Figure 3. The dark area near the tip of the leaf was produced by infection of mildew. ×4½

FIGURE 3.—Typical stippling produced by *E. bifurcata* on *Solidago* sp., one of its preferred hosts. Note the normal colored areas in the center of many spots; also the slightly coarser stippling than in Figure 2. The feeding on leaves in Figures 2 and 3 was produced by several adults during an undetermined interval of time. ×4½

73318—31——6

normal-appearing centers of the stippled spots showed uninjured palisade cells directly above the point of entrance of the beak in the lower epidermis. (Fig. 4.) The leaf hopper is apparently unable to puncture the plant at right angles to the long axis of its body and it

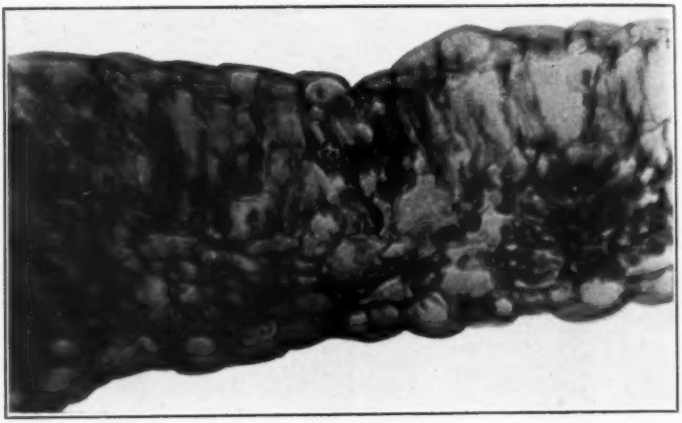


FIGURE 4.—Section of alfalfa leaf through center of stippled area produced by feeding of *E. bifurcata* on the lower surface. Cells in the spongy mesophyll and in the palisade layer have been emptied of their contents. Observe group of uninjured palisade cells directly above the point of puncture. × 315

can not therefore reach the tissue directly above the point of puncture. *E. abrupta* reached an area from 10 to 15 palisade cells in diameter in the mesophyll from one puncture; the other species of this group reached an area of from 6 to 12 cells from one puncture. When

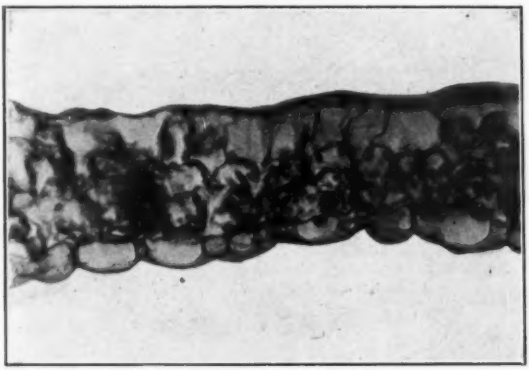


FIGURE 5.—Section of alfalfa leaf through portion of stippled area produced by feeding of *E. erigeron*. Note the collapse of the upper epidermal cells. × 315

punctures are made close together the areas of destroyed mesophyll become confluent. It appeared from the sections studied that *maligna*, *erigeron*, and *bifurcata* tend to feed on the spongy mesophyll as well as on the palisade cells. (Figs. 4 and 5.) The palisade cells

seem to be preferred by *filamenta* and *abrupta*. (Fig. 6.) In many stippled areas, however, exceptions may be found. Stained sections of the exposed stems and petioles showed punctures through the cortex and these cells were emptied of their contents. The phloem in the stems and petioles did not appear to attract these species, although the vascular bundles were penetrated in a few instances. In both the stems and the leaves sheath material that stained bright red with safranin was found in irregular accumulations in the middle lamella between the cells of the cortex or of the mesophyll. Only occasionally were cells observed that were either partially filled or surrounded with this material.

The stippled areas are located irregularly over the leaves, usually at some distance from the midvein or the lateral veins, but occasionally directly over them. Sections through the stippled areas about the veins, studied microscopically, showed the greatest accumulation of sheath material around the bundle. In some instances it was found

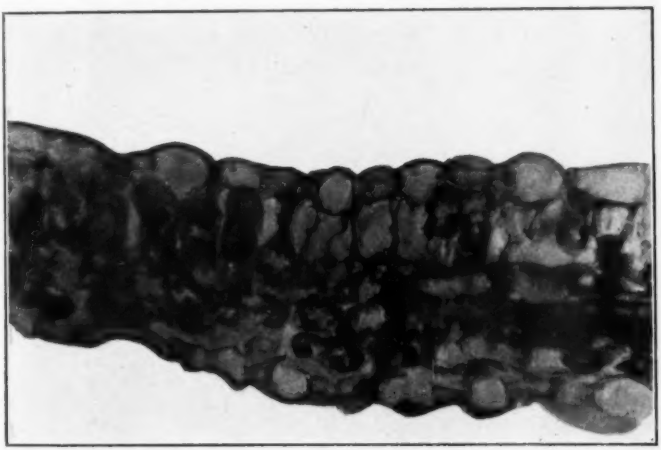


FIGURE 6.—Section through portion of stippled area produced by *E. filamenta* on alfalfa leaflet. × 315

that the stylets of *filamenta* had penetrated directly through the phloem and xylem intercellularly to the mesophyll above. The veins seem to interfere with the normal feeding of the leaf hoppers of this group, and no evidence was found that they were searching for the phloem as a source of food. (Fig. 7.)

Horne and Lefroy (*5, p. 385*) [1] described the spotting of foliage by the leaf hoppers *Eupteryx atropunctata* Goeze and *Chlorita viridula* Fall. They found that the assimilatory tissue was destroyed in the spotted areas but without killing the plant. Smith (*10, p. 119–121*), in his studies of the leaf hoppers *Eupteryx auratus* Liv. and *Zygina pallidifrons* Edw., found that the mesophyll tissue was attacked and that "spotting" of the foliage resulted. He stated that the spots enlarged after the leaf hoppers were removed, and he concluded that the salivary secretions gradually diffused through the cell walls

[1] Reference is made by number (italic) to Literature Cited, p. 284.

without injuring them but that they decolorized and destroyed the chlorophyll. In the present studies stippled areas on young leaves were found to enlarge slightly as the leaf expanded. With the binocular microscope careful measurements were made of stippled spots on mature alfalfa leaves at 2-day intervals for 14 days after the leaf hoppers had been removed. These measurements failed to show any enlarging of the areas. Further, the green centers of the stippled areas and the small groups of cells forming salients into these areas did not disappear during the period of these observations. This seems to indicate that the injury caused by these species of Empoasca is largely mechanical and that a toxin, if injected, does not make itself apparent.

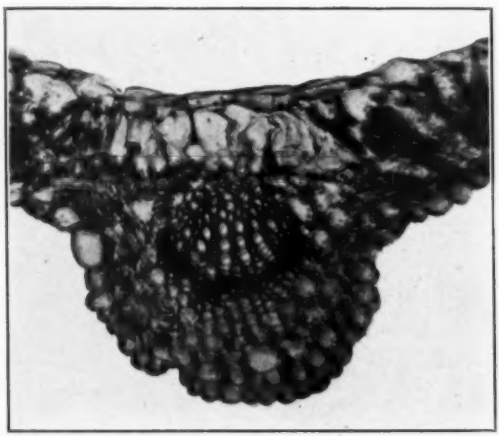


FIGURE 7.—Section through stippled area above midvein of alfalfa leaf produced by *E. bifurcata*. The mouth parts entered the leaf to the right of the midrib and extended to the palisade cells above the vascular bundle. The cells of the bundle and the parenchymal tissue immediately surrounding them appear to be normal. Compare with Figure 14. × 315

GROUP 2.—PRIMARILY PHLOEM FEEDERS

Group 2 includes *Empoasca fabae* (Harris) from Virginia and strains from other States widely separated, which resemble each other morphologically. All of these will be considered as one species in the present paper. Several workers have shown that *E. fabae* produces hopperburn of potato and a diseaselike injury to alfalfa and many other plants. When this leaf hopper is confined on tender stems or petioles of apple, alfalfa or clover (fig. 8), wilting of the plant parts above the point of feeding occurs. When it is confined on the lower surface of the leaflet the midvein and lateral veins become lighter in color and wilting takes place in one or two days. When there is less feeding, wilting does not take place but the typical symptoms of tipburn, or of reddening or yellowing of leaves, according to the host plant, appear after a longer interval of time. Usually there is no evidence of stippling on the upper surface (see normal leaf, fig. 9), but occasionally very indistinct stippling, like small

scratches, is apparent along the veins or in the mesophyll between the veins. (Fig. 10.)

A microscopic examination of sections through the leaves showed that the cells of the phloem are frequently punctured and are torn and distorted (reported as disorganized by Granovsky (*4*) or are filled, partially or completely, with the red-stained sheath material. The stylets of the insect pass intercellularly through the parenchyma of the midvein and the path is marked by an ill-defined sheath. Evidently the leaf hopper in search of fresh phloem tissue makes exploratory punctures in the neighborhood of the bundles of the main

FIGURE 8.—Two wilted leaves of Russian red clover each of which has been fed upon between the two black marks on the petioles for 48 hours by one adult of *E. fabae*. × 2

vein or its laterals and even reaches the epidermis on the opposite side of the leaf. (Fig. 11.) The cells of the spongy mesophyll and palisade layers in these areas are emptied of their contents and this produces the scratchlike stippling which is apparent on the upper surface. While the mesophyll cells are fed upon by *fabae* along the lateral veins, only one to three or four cells are fed upon about a single puncture (fig. 12), in contrast with the many cells fed upon by the species of Group 1. The sheath material observed between the cells and in the intercellular spaces was more abundant in the areas fed upon by *fabae* than in those fed upon by the members of the first group. (Fig. 11 and 12.) Stained sections through the petioles

FIGURE 9.—Normal leaflet of alfalfa. × 5

FIGURE 10.—Alfalfa leaflet showing the fine scratch-like stippling produced by an adult *E. fabae* during 24 hours' feeding. Most of the scratches are near the veins and differ from the feeding areas in Figure 1. × 4½

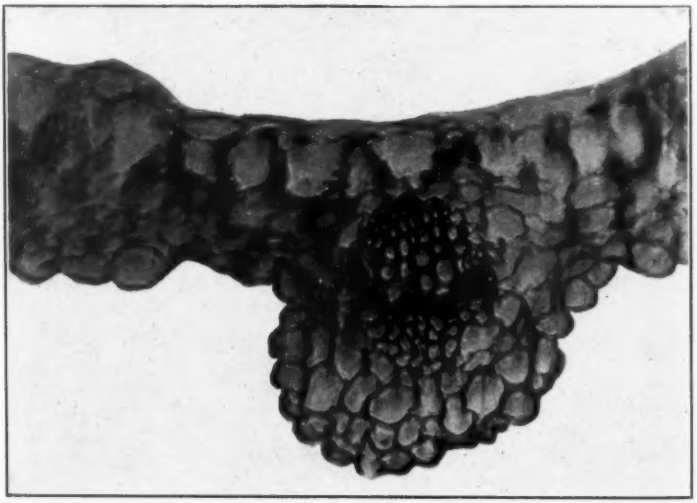


FIGURE 11.—Midrib of an alfalfa leaf which had been fed upon from the lower surface by an adult of *E. fabae*. The phloem is torn and intermixed with sheath material while the xylem vessels are not altered. The palisade cells above and to the left of the bundle have been fed upon and a considerable amount of sheath material is present. Note the shrinking of the tissue in the area of destroyed mesophyll. × 210

and stems showed that the phloem is the tissue usually sought by the leaf hopper. Here again the phloem cells were disorganized by being torn and by having the sheath material located intercellularly and

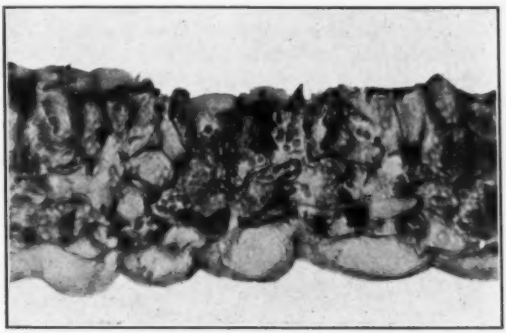


FIGURE 12.—Section through two stippled areas in mesophyll of alfalfa leaflet which was fed upon by *E. fabae*. Only a few palisade cells have been emptied of their contents in each area. A large amount of heavily stained stylet sheath material is located between some of the empty cells and also in close contact with normal-appearing cells. × 315

intracellularly. The cell walls of the cortex in these plant parts seem to collapse less readily and the well-defined cylindrical sheaths are obtained in sections. (Figs. 13 and 14.) In most of the sections

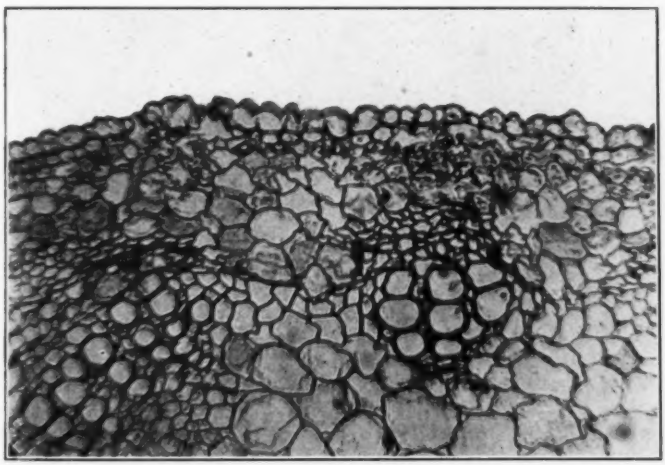


FIGURE 13.—Section through stem of alfalfa showing stylet sheath of *E. fabae* leading intercellularly through the cortex to the vascular bundle. The sheath is continuous across a large intercellular space in the cortex. The phloem cells in this bundle are torn, intermixed with sheath material, and shrunken as is seen by comparison with the normal bundle to the right. × 200

from unwilted tissue the xylem had evidently not been penetrated in the bundles of the stem, petiole, or midvein. In a few of the lateral veins sheath material was found between the xylem tubes and

in close proximity to this group of cells. Because of the collapse of the cells, the early studies of the tissue which had wilted after exposure to adults of *fabae* resulted in a failure to determine which tissues they had fed upon. However, in later studies it was found possible to restore the flaccid or moderately wilted tissue to a condition approaching its original turgidity by submerging the small pieces desired for sectioning in distilled water for one or two hours before proceeding with the usual fixation, dehydration, and embedding. In exposed portions of midribs of leaves, and in slender, succulent young petioles and stems of alfalfa, Russian red clover, Ladino clover, and potato, which had wilted and were treated as above mentioned, positive evidence of plugging in the xylem tubes was found by sectioning and staining. (Figs. 15, 16, and 17.) The presence of this sheath

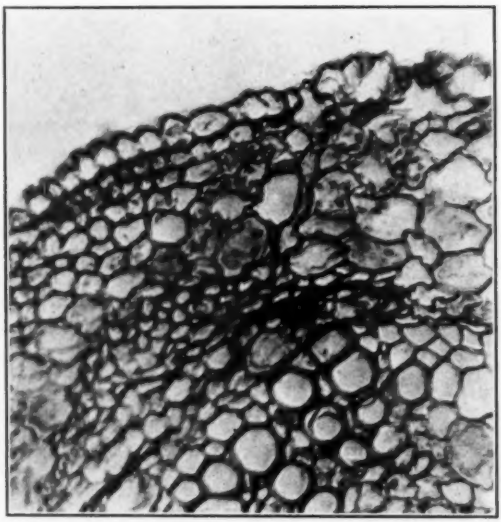


FIGURE 14.—The disorganized phloem of the vascular bundle, the cortical area, and the stylet sheath of Figure 13 enlarged to give more details. × 315

material was also shown by microchemical tests to be present in the water-conducting cells.

Smith (*10*) found that the stylet sheath of the following insects was definite in outline and that it followed an intercellular path: White fly, *Asterochiton vaporariorum* Westw.; coccids, *Aspidiotus hederae* Vallot and *Dactylopius longispinus* Targ.-Tozz.; and aphids, *Myzus persicae* Sulz., *M. circumflexus* Bukt., and *Macrosiphum solanifolii* Ashm. All, except *A. hederae*, which reached only into the cortex, fed on the cells in the vascular bundle. Horsfall (*6*) found that the path of the proboscis of *Aphis rumicis* L. and of *Myzus persicae* followed as a rule the middle lamella through the epidermis and cortex or mesophyll. Apparently the objective of the beak of *M. persicae* is the vascular bundle, where usually the phloem cells, but

also occasionally the tracheal tubes, are pierced and plugged. Davidson (*1*) also found definite setal sheaths of the three species of aphids which he studied. Wardle (*11*) did not mention the presence of a feeding sheath in tissue which had been fed upon by *Thrips tabaci*. Woods (*12, p. 14*) described the setal sheath produced by *Rhopalosiphum dianthi* Schrank and noted the gradual enlarging of the spots about the feeding puncture in carnations.

Davidson (*1, p. 45*) found that the contents of the cells which were in contact with the saliva along the path of the stylets of *Aphis*

FIGURE 15.—Midrib of alfalfa leaf, which had been fed upon by an adult of *E. fabae*, showing sheath material between the parenchymal cells at the lower right of the bundle, plugged phloem, and all except two of the xylem vessels filled with the sheath material. × 430

rumicis were extensively plasmolyzed and exhibited a migration of the disorganized cytoplasm toward the source of irritation. With *A. rumicis* there was no spreading to surrounding tissue but with *Myzus cerasi* Fab. on peach and *Macrosiphum rosarum* Walk. on rose many cells were torn and destroyed. Horsfall (*6, p. 10–11*) found that in sections of leaves fed upon by *Aphis rumicis* cells contiguous to the setal sheath were normal, while in other sections plasmolysis, similar to that observed by Davidson, had taken place. Painter (*8, p. 505*), working with *Psallus seriatus*, found that marked plas-

molysis took place about its feeding punctures and that the entire cell contents became decidedly homogeneous.

The feeding puncture of *Empoasca fabae* generally follows an intercellular path and resembles the punctures of the insects studied by Smith, Horsfall, Davidson, and Woods. However, no evidence has so far been found that the cells surrounding the puncture made by *E. fabae* were plasmolyzed, nor was there any apparent thickening of cell walls such as was observed by Davidson with *Macrosiphum rosarum* on rose. As with the stippling caused by species of Empoasca in Group 1, the scratchlike stippling produced by *fabae* did not seem to enlarge after the leaf hopper had been removed.

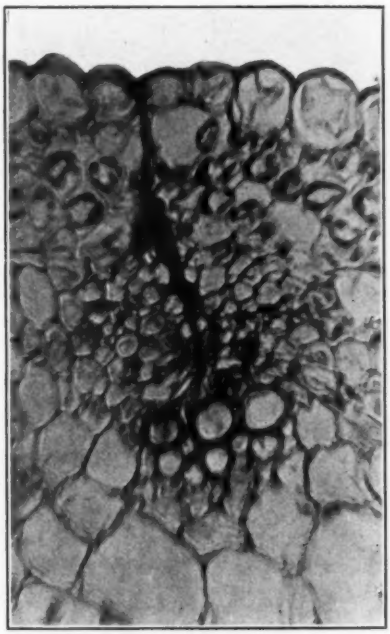


FIGURE 16.—Stem of alfalfa showing sheath material of a feeding puncture of an adult *E. fabae* from the epidermis through the cortex into the vascular bundle with two of the large xylem vessels plugged. ×430

The injury produced by *Empoasca fabae* is extremely localized, affecting only that part of the plant beyond the point of feeding, whether the injury is manifested as wilting or as yellowing or reddening. This fact when considered in relation to the feeding habits of this insect suggests that the disorganization of the phloem elements may result in a disturbance of the plant processes above the point of attack and that the production of substances by the plant itself may cause the partial (yellowing or reddening) or complete breakdown (potato hopperburn) of the tissues involved. This hypothesis is strengthened by the evidence shown in Figure 18. The sudden swell-

ing at the upper end of the feeding area, the increased diameter and the development of the purplish pigmentation of the stem above, and the modification of the laterals, all point to the piling up of materials in these parts owing to interference with their normal downward movement in the phloem. Were a toxin being injected by the leaf hopper the symptoms would probably not have been manifested in

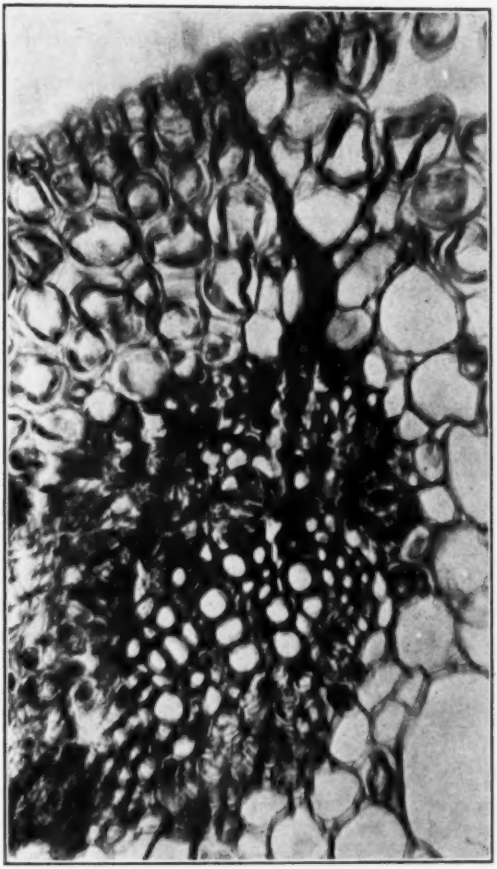


FIGURE 17.—Petiole of Russian red clover showing a feeding puncture by an adult of *E. fabae* through the cortex into a vascular bundle where the phloem is disorganized and where several of the xylem vessels have been plugged. × 430

this manner. The same individual that produced this abnormal condition caused wilting in a previous exposure of 24 hours on a young alfalfa tip. The wilting of the plant part beyond the point of feeding may be explained by the fact that many of the xylem vessels were plugged. The present studies indicate that this explanation is more

likely to be correct than the hypothesis of toxin injection advanced by other workers (*2*, *3*). It is quite certain that the toxin, if one is introduced by *fabae*, is less positive in its reaction than that found in the feeding punctures of the capsids *Plesiocoris rugicollis* Fall. and *Lygus pabulans* L. (*9*).

The behavior of *Empoasca fabae* differed markedly from that of any of the other five species studied. Adults of species in Group 1 were able to live on slightly wilted foliage for some time, whereas *fabae*, when confined to this type of host material, died almost as quickly as if no food had been provided. When confined to the upper surface of the alfalfa leaf the adults of *fabae* usually lived only 4 to 10 hours. When confined to an area 15 mm. in diameter on the lower surface, the adults died unless they were transferred to a new leaflet at least once a day; if transferred regularly each day, they lived a maximum of 3 to 4 days. If confined in small cages 15 mm.

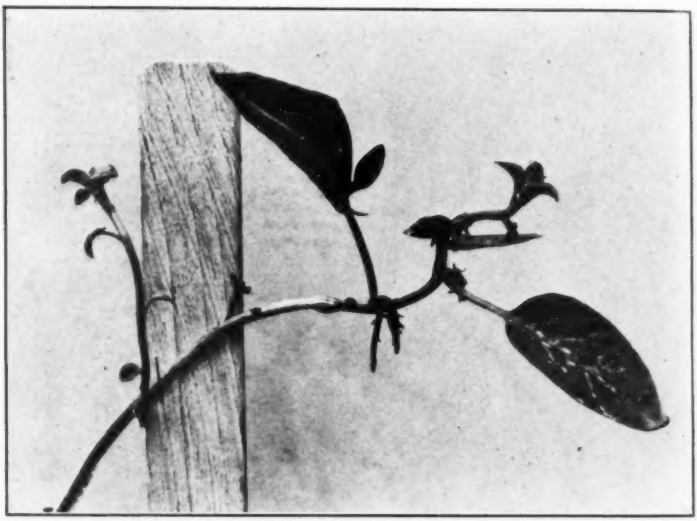


FIGURE 18.—A shoot of potato showing an enlargement of the stem above the point of feeding by *E. fabae*. A single adult was confined for 9 days between the two black marks on the stem. Photographed 20 days after the first feeding of the leaf hopper. × 1

in diameter, upon tender stems or petioles, the adults usually lived 2 or 3 days without change to new stems or petioles, but if changed regularly every day they lived a maximum of 14 days. The longest records of adult life were obtained from individuals confined on the tender petioles of cowpeas. In a few tests adults died soon after being confined to large, but apparently succulent, stems of potato near the top of the plant; others confined upon the petioles and leaf veins lived longer. Sections of the stems showed feeding punctures into the cortex, but apparently the cortical layer was too thick for the adults to reach the phloem with their mouth parts and therefore death resulted. The phloem tissue was reached in the petioles and in the leaf veins.

DISCUSSION

The injury to the host plant produced by members of Group 1 is localized and, comparatively speaking, is not serious. The destruction of the mesophyll in the limited areas undoubtedly reduces the photosynthetic activity of the leaves in proportion to the size of the areas destroyed. However, the adjacent remaining uninjured areas retain their color and apparently their normal functions. The feeding by *Empoasca fabae* on the phloem or water-conducting tissue is much more serious in its results. One of the species in group 1 may make a dozen or more stippled areas in the mesophyll of a group of leaflets without seriously injuring them, but a single adult of *E. fabae* making approximately an equal number of feeding punctures in the petiole of a similar leaf will cause injury to the entire leaf. A study of serial sections of a cowpea petiole on which a single adult of *E. fabae* had fed for 24 hours showed that 14 punctures into the phloem had been made and that 5 of the 7 bundles in the petiole had been fed upon. These results are in marked contrast with those obtained in feeding tests with *Gypona octolineata* (Say) and *Aphis rumicis*. An individual of *G. octolineata*, after feeding for three weeks on a tender apple stem apparently made only one puncture into the vascular tissue, as shown by studies of serial sections of this plant material. Davidson (*1, p. 48*) confined four apterous individuals of *A. rumicis* for 24 hours on localized areas of young bean plants and found only four stylet sheaths in the sections. In most feeding punctures of *E. fabae* whenever the phloem was reached all or nearly all of the phloem cells in that bundle were ruptured or otherwise disorganized. This disorganization of one of the tissues of translocation in the plant is much more serious than the disorganization of an equal amount of mesophyll tissue. The feeding in the xylem and the plugging of the tubes there evidently result in an equally serious injury, that is, wilting beyond the point of feeding, on certain hosts. Le Clerg and Durrell (*7, p. 15*) showed that the water flow in the vascular bundles of crowns of wilt-infected alfalfa plants was reduced 33 per cent as compared with that in crowns of healthy plants. The striking symptoms of wilting and dwarfing, occurring in wilt-infected plants which probably contained the same amount of plugging, are illustrated by these authors (*7, p. 5*). While accurate measurements of the proportionate amount of plugging of xylem vessels by *E. fabae* have not been made, in some instances more than one-third of the vessels seem to have been plugged. (Fig. 15.)

The feeding of the two groups of leaf hoppers, the one upon the mesophyll and the other upon the phloem, or xylem, is evidently not a matter of choice on the part of the insects. Instead it seems necessary, physiologically, for the life of the species that it procure its particular type of food from a definite region in the host plant. The phloem of rapidly growing plant parts is naturally rich in nutritive substances such as the albuminoids of the amino acid type, carbohydrates as sugars, and inorganic salts, as stated by Davidson (*1, pp. 49, 50*). These are all in soluble form and probably require little or no digestive changes before they are assimilated by the leaf hopper. The chloroplasts and other materials in the cytoplasm of the mesophyll cells present a very different type of food material. Whether *Empoasca fabae* has digestive enzymes unlike those of the other species

which would account for this difference in feeding habits, has not been determined.

SUMMARY

On the basis of the results obtained in a study of the feeding habits of six species of Empoasca the species were divided into two groups. The first group included *maligna*, *abrupta*, *filamenta*, *bifurcata*, and *erigeron*. This group is characterized by a habit of feeding on the mesophyll tissue of the leaves and the regular production of definite spotting or stippling on the upper surface. The more mature foliage seems to be preferred to that of the more succulent, younger leaves. Strains of *E. fabae*, which constitute the second group, evidently feed by preference upon the phloem or water-conducting tissue, and the well-being of this species seems to depend upon the availability of fresh phloem tissue in succulent plant parts.

When *Empoasca fabae* is confined to mesophyll tissue, and the mesophyll-feeding species of Group 1 are confined to succulent phloem tissue on growing tips and petioles, all die within a short time. These differences in feeding habits seem to be correlated with the physiology of the species.

The feeding by *Empoasca fabae* in the phloem tissue or xylem vessels results in more serious injury to the host plant than does the feeding by an equal number of individuals of the other species of Empoasca on the mesophyll tissue.

No evidence was found that a toxin was introduced into the plant by any of the species. On the contrary, the present studies indicate that injury by *Empoasca fabae* is the result of interference with translocation of plant materials which produces either wilting when xylem vessels are plugged or yellowing or reddening when the phloem is disorganized and plugged.

LITERATURE CITED

(1) Davidson, J.
1923. Biological studies of aphis rumicis linn. the penetration of plant tissues and the source of the food supply of aphids. Ann. Appl. Biol. 10: [35]–54, illus.

(2) Eyer, J. R.
1922. Notes on the etiology and specificity of the potato tip burn produced by empoasca mali le baron. Phytopathology 12: [181]–184, illus.

(3) Fenton, F. A., and Hartzell, A.
1923. Bionomics and control of the potato leafhopper empoasca mali le baron. Iowa Agr. Expt. Sta. Research Bul. 78, p. [379]–440, illus.

(4) Granovsky, A. A.
1930. Differentiation of symptoms and effect of leaf hopper feeding on histology of alfalfa leaves. (Abstract) Phytopathology 20: 121.

(5) Horne, A. S., and Lefroy, H. M.
1914. Effects produced by sucking insects and red spider upon potato foliage. Ann. App. Biol. 1: 370–386, illus.

(6) Horsfall, J. L.
1923. The effects of feeding punctures of aphids on certain plant tissues. Penn. Agr. Expt. Sta. Bul. 182, 22 p., illus.

(7) Le Clerg, E. L., and Durrell, L. W.
1928. Vascular structure and plugging of alfalfa roots. Colo. Agr. Expt. Sta. Bul. 339, 19 p., illus.

(8) PAINTER, R. H.
1930. A STUDY OF THE COTTON FLEA HOPPER, PSALLUS SERIATUS REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES. Jour. Agr. Research 40: 485–516, illus.

(9) SMITH, K. M.
1920. INVESTIGATION OF THE NATURE AND CAUSE OF THE DAMAGE TO PLANT TISSUE RESULTING FROM THE FEEDING OF CAPSID BUGS. Ann. Appl. Biol. 7: 40–55, illus.

(10) ———
1926. A COMPARATIVE STUDY OF THE FEEDING METHODS OF CERTAIN HEMIPTERA AND OF THE RESULTING EFFECTS UPON THE PLANT TISSUE, WITH SPECIAL REFERENCE TO THE POTATO PLANT. Ann. Appl. Biol. 13: 109–139, illus.

(11) WARDLE, R. A., and SIMPSON, R.
1927. THE BIOLOGY OF THYSANOPTERA WITH REFERENCE TO THE COTTON PLANT. PART 3. RELATION BETWEEN FEEDING HABITS AND PLANT LESIONS. Ann. Appl. Biol. 14: 513–528, illus.

(12) WOODS, A. F.
1900. SIGMONOSE: A DISEASE OF CARNATIONS AND OTHER PINKS. U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 19, 30 p., illus.

U. S. GOVERNMENT PRINTING OFFICE: 1931

XUAN

XU